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Roentgen Stereophotogrammetric
Analysis of Cranial Vault
Growth in Rabbits

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Analysis of Cranial Vault
Growth in Fetalis



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The present summary is based on the following papers. They will be referred to by their Roman numerals (I - VII).

- I. Selvik, G., Alberius, P., Aronson, A.S.: A roentgen stereophotogrammetric system. Construction, calibration and technical accuracy. - Accepted in *Acta Radiol. (Diagn.)*.
- II. Alberius, P.: Bone reactions to tantalum markers. A scanning electron microscopic study. - *Acta anat.* 115:310-318, 1983.
- III. Alberius, P., Selvik, G.: Roentgen stereophotogrammetric analysis of growth at cranial vault sutures in the rabbit. - Accepted in *Acta anat.* (Vol. 117, 1983).
- IV. Alberius, P., Selvik, G.: Kinematics of cranial vault growth in rabbits. - Accepted in *Am. J. Anat.*
- V. Alberius, P., Selvik, G., Ekelund, L.: Roentgen stereophotogrammetric analysis of neurocranial suturectomy in rabbits. - Submitted for publication.
- VI. Alberius, P., Selvik, G.: Roentgen stereophotogrammetric analysis of restricted periods of neurocranial sutural immobilization in rabbits. - Submitted for publication.
- VII. Alberius, P.: Pattern of membranous and chondral bone growth. A roentgen stereophotogrammetric analysis in the rabbit. - *Acta anat.* 116:37-45, 1983.

CONTENTS

INTRODUCTION

REVIEW OF LITERATURE

Growth at cranial vault sutures	8
Sutural morphology	9
Craniosynostosis	10

PURPOSES OF THE PRESENT INVESTIGATION	12
---------------------------------------	----

DEFINITIONS AND ABBREVIATIONS	13
-------------------------------	----

Comment on nomenclature	15
-------------------------	----

MATERIALS AND METHODS

Animals	16
Bone markers	16
Implantation instrument	16
Bone marker positioning	18
Bone marker requirements	18
Roentgen stereophotogrammetric equipment	19
Exposure procedures	21
Film and computer analysis	21
Volumetric registrations	22
Kinematic calculations	22
Technical accuracy	23
Operative technique	23
Suturectomy	24
Temporary sutural immobilization	24
SEM procedure	24
Macroscopic investigation	25
Exposure dose measurements	25

RESULTS

Technical aspects on RSA	26
Bone reactions to tantalum markers	26
Post-implantation cranial vault growth	28
Neurocranial suturectomy	29
Restricted periods of sutural immobilization	31
Comparison of membranous and chondral bone growth	32

GENERAL DISCUSSION

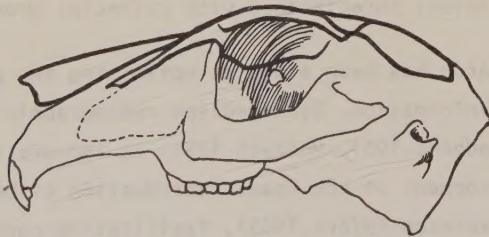
Methodological aspects	35
Cranial vault growth	36
Growth after trauma to the coronal suture	39

GENERAL SUMMARY	42
-----------------	----

ACKNOWLEDGEMENTS	44
------------------	----

REFERENCES	45
------------	----

PAPERS I - VII



INTRODUCTION

Calvarial bones arise at widely separated ossification centers and spread centrifugally towards each other until bone growth is halted at the sites of major reflections in the dura mater. The mammalian dura is firmly attached to the cranial base at five principal points (crista galli, lesser sphenoidal wings and petrous crests), from which fiber tracts run upwards to the cranial vault hereby resulting in the calvarial suture system (reviews: Moss 1975, Smith and Töndury 1978, Friede 1981). These sutures remain unossified connective tissue regions for varying periods of time.

An expanding interest in the field of skull growth has occurred since Tessier developed new treatments of major cranial disorders, including severe craniosynostosis (craniostenoses) and faciostenoses (1971 a,b). Still, surgery in these regions is not completely developed, often giving suboptimal postoperative results. This calls for new approaches in cranial research. A detailed knowledge of normal cranial growth mechanisms, the effect of external influences and adaptive growth capacities is therefore required.

Skull growth has been studied by a wide variety of methods (methodological review: Sarnat 1963). Roentgenographic registration offers many advantages, e.g. possibilities of longitudinal growth analysis, easy handling and a minimal interference with potential growth sites.

Much research has been aimed at optimizing the procedure for registration of such information. Standardized radiographic cephalometry was developed (Broadbent 1931, Hofrath 1931) to improve the assessment when studying the development of the head. Introduction of bone markers into the human facial skeleton (Björk 1955), facilitating correct reorientation, was a further step forward. This technique has long been used experimentally to obtain skeletal landmarks when distinct anatomical marks are unavailable (review: Sarnat and Selman 1978).

To define spatial positions, a totally different approach is needed. Davidson and Hedley (1897) as well as Payne (1897) described principles for three-dimensional (3-D) localization from radiographs two years after Röntgens discovery of "x-rays". Since then, many attempts to spatially reconstruct objects have been attempted. No systematic use of roentgen stereo measurements seems to have occurred until the 1970's, when several techniques were developed. In 1974, Selvik published a method for roentgen stereophotogrammetric analysis (RSA) using metal indicators, roentgen calibration systems and comprehensive computer software.

To determine short period skull growth exact measuring techniques are required. By the introduction of the highly accurate RSA new aspects on the biomechanical events in cranial growth may be obtained. The rabbit was chosen in this investigation since it has frequently been used in comparative studies on cranial growth, and its skull bone size is sufficient for optimal control of marker stability.

Growth at cranial vault sutures

In cranial morphogenesis, three types of bone growth have been described: periosteal, sutural and enchondral bone formation. Normally, only the first two apply to intramembranous bones.

The multifunctional role of the neurocranial sutures is undisputed. During growth they participate in spatial adjustments between calvarial bones. Furthermore, these joints absorb shocks and prevent any undue separation. Their adaptability is also demonstrated by the fact that experimental trauma may result in variations in sutural location and morphology (Moss 1954, Girgis and Pritchard 1958, Sarnat 1958, Sirola 1960, Ryöppy 1965). Recently, a restraining growth-modulating function has been discussed (Persson 1973, Persson et al. 1979, Babler et al. 1982a).

Today, there exist two main philosophies regarding the control of sutural growth. One considers the sutural tissue to possess autonomous growth potential (Moore 1949, Weinmann and Sicher 1955, Prahl 1968). Contrary to this, the opposite, predominant, philosophy regards the sutures to grow in response to factors outside the suture proper. Thus, bone deposition along the sutural borders is considered a compensatory process, secondary to bone separation resulting from growth in other systems.

One such theory of great importance both to neurocranial and craniofacial research was introduced two decades ago. This "functional matrix" theory was developed by Moss from the concepts of van der Klaauw (1952). According to Moss all functional cranial components (skeletal units and functional matrices) are maintained within cranial capsules. The neurocranial capsule surrounds and protects its "capsular matrix", consisting of the brain, the leptomeninges, and the cerebrospinal fluid, i.e. the matrix is identical to the neural mass volume. The form and spatial location of components included in the neurocranial capsule is determined primarily by the volumetric demands of the enclosed capsular matrix (Moss

and Salentijn 1969). Others, though, suggest growth in the cranial cartilages to be partly or completely responsible for sutural bone separation (Scott 1954, Baume 1961, van Limborgh 1970, 1972). Experimental and clinical data on these issues have been extensively reviewed elsewhere (e.g. Hoyte 1966, Rønning 1971, Linge 1973, Persson 1973, Alhopuro 1978).

Sutural morphology

Sutural architecture is believed to vary both in man and animals, for which many more or less speculative explanations have been put forward. Moss (1957) suggested the butt (or flat) appearance to be the basic (or intrinsic) sutural area morphology. Bones adjacent to flat sutures are not subject to any essential separating forces (Moss 1958a). The basic type is illustrated by the anterior sagittal suture, which is stabilized by the nasal septum and the perpendicular plate of the ethmoid bone. Furthermore, as pointed out by Roskjaer (1977), the rabbit nasal bones have no muscle insertions.

The bevelled type (or overlapping or squamous) is supposed to be a functional derivative, resisting disarticulation, absorbing stress and allowing adjustive movements (Moss 1957, Gans 1960, Herring 1972) as well as making faster separation possible (Linge 1973, Koskinen et al. 1976, Koskinen 1977). This type is represented by the transverse sutures of the rabbit vault.

Both types may be modified during growth by secondarily developed interdigitations, due to functional demands (Troitzky 1932, Massler and Schour 1951, Simpson et al. 1953, Moss 1957, 1961, Young 1959, Isotupa et al. 1965, Smith and McKeown 1974, Koskinen et al. 1976, Koskinen 1977, Oudhof 1978, Foley and Kokich 1980). These interdigitations serve to increase the area (Noyes 1934, Gans 1960, Herring 1972), transmit tension (Herring 1972), enable stress resistance (Gans 1960, Herring 1972), and help to maintain a functional distance between the bones (Koskinen et al. 1976). Possibly they indicate the general direction of bone separation (Isotupa 1972, Koskinen et al. 1976) but this is not agreed upon by Linge (1973). Also,

sutural morphology is determined by specific growth patterns and biochemical characteristics (Persson 1973).

Craniosynostosis

Although Hippocrates in 400 B.C. described premature closure of the cranial sutures (cited by McCarthy 1979, Ferguson 1980), its scientific study did not begin until the middle of the 19th century. The description and systematic classification of vault deformities associated with premature sutural fusion was originated by Virchow (1851). Some years later, Lannelongue (1890) introduced linear craniectomy in operating on a patient with microcephaly. Poor results led to abandonment of the treatment until Faber and Towne (1927) revived surgical intervention to prevent blindness and other complications, and they also stressed the importance of surgery in early infancy. Since then various surgical techniques have been described. A considerable advance was made by Tessier, who introduced new principles in treatment of major deformities like Crouzon's disease and Apert's syndrome (1971a, b).

Because of the rapid regrowth of bone across the craniectomy site, the use of foreign materials along its margins has been recommended to avoid resynostosis and achieve sutural patency. Simmons and Peyton (1947) and Ingraham et al. (1948), respectively, covered the craniectomy edges with tantalum foil or polyethylene film, somewhat unsuccessfully. Anderson and Johnson (1956) applied Zenker's solution, a tissue fixative, to the dura mater. The initially observed side-effects can now be avoided by using modified Zenker's solution (glacial acetic acid excluded), reducing the time and amount of application, avoiding the superior sagittal sinus, and by careful rinsing of the surgical site (Alberius, Brandt and Selvik, unpublished data, 1983).

The embryopathology involved in craniosynostosis is still poorly understood. Nevertheless, there are two probable mechanisms; 1. the sutural fusion is a primary defect, resulting in secondary cranial base abnormalities, or 2. the premature closure of a calvarial suture manifests a

basal bone malformation.

Moss (1959) speculated that, due to basicranial abnormalities, there is a malrelation of the points of dural attachments and the sutural origins, causing abnormal tension to be transmitted through the dura, thereby resulting in premature fusion. There is now some clinical data supporting this view (Stewart et al. 1977, Hahn and Jane 1978). Albright and Byrd (1981), on the other hand, contended that pathological cranial base changes do not require the presence of dural traction bands as the primary defect may be ossification of basal sutures initially altering the skull base, and thereafter progressing superiorly to the calvarial sutures (Simmons and Peyton 1947, Anderson and Geiger 1965, Shillito and Matson 1968, Davis et al. 1969, Seeger and Gabrielsen 1971, Gates and Dore 1975).

Nevertheless, premature closure of the coronal suture in man is a major trauma to normal skull expansion, due to the sutures continuity in the skull base. The growth here normally constitutes such a large percentage of the entire skull growth that the remaining sutures are unable to compensate (Bertelsen 1958), resulting in disturbed coordination of cranial vault and facial skeletal growth rates (Moss and Greenberg 1955, Tressera and Fuenmayor 1981). This also applies to the rabbit, whose coronal suture similarly converges with sutures of the cranial base, thus making it suitable for experimental testing of surgical innovations.

In conclusion, cranial development is still obscure. Malformations of the cranial vault and the facial skeleton, often associated with basicranial disturbances, call for extended surgery, whose exact design and extent consequently are not yet fully known.

PURPOSES OF THE PRESENT INVESTIGATION

- to describe the construction, calibration and technical accuracy of the roentgen stereophotogrammetric system used
- to assess the biocompatibility of tantalum bone markers
- to apply roentgen stereometry to experimental studies on the rabbit calvarium in order to further elucidate and characterize sutural growth and bone rearrangements
- to investigate sutural responses as well as compensatory and volumetric consequences of two types of trauma:
 - coronal suturectomy
 - restricted periods of sutural immobilization
- to study relations between intramembranous and enchondral bone growth.

DEFINITIONS

Accuracy:	a measuring procedures closeness to the true value.
Calibration cage:	the glass-plexiglass equipment holding tantalum markers of known internal positions, for geometrical determination of the bundles of roentgen rays. The tantalum markers define the laboratory coordinate system.
Control points:	the indicators of the upper plate of the calibration cage, utilized to determine the positions of the two roentgen foci in the laboratory coordinate system.
Coordinate system:	a right-hand orthogonal (Cartesian) coordinate system is used.
Displacement:	the relation between the actual position of a moving object and its reference position.
Fiducial marks:	the indicators of the lower plate of the calibration cage, used to transform film coordinates to laboratory coordinates.
Kinematics:	the mathematical description of motion regardless of the cause of motion.
Mean error of rigid-body fitting (e_{rb}):	the minimal mean distance between segment indicators moved in a rigid-body motion compared to their actual motion.
Motion/movement:	positional change relative to a reference system.
Precision:	a measure of the dispersion of values about a mean. High precision = low dispersion.
Projective transformation:	eight-parameter, fractional linear, transformation. An analytical expression for central projection (central perspective) between planes.

Rigid body:	an object for which internal distances remain constant in time.
Rigid-body model:	a configuration of three or more non-collinear markers, stable relative to the rigid body. In the present context the markers are implanted in a bone, assumed rigid.
Roentgen stereophotogrammetry:	a 3-D reconstruction of objects from measurements of radiographs.
Rotation:	a displacement when markers on a line (the rotation axis) remain unchanged, but markers outside this line move relative to their distances from the line.
Similarity transformation:	four-parameter transformation. A 2-D transformation consisting of a rotation, a shift, and a scale change.
Translation:	a parallel displacement of all markers constituting a rigid body or, for one marker, a positional change.
Total translation:	the length of the translation vector.

ABBREVIATIONS

CP	=	control point
e_{rb}	=	mean error of rigid body fitting
FM	=	fiducial mark
PT	=	projective transformation
RBM	=	rigid-body model
RSA	=	roentgen stereophotogrammetric analysis
SEM	=	scanning electron microscopy
ST	=	similarity transformation
2(3)-D	=	two(three)-dimensional

COMMENT ON NOMENCLATURE

For reasons of simplicity the sutures studied are referred to as calvarial or cranial vault sutures, despite both the internasal and fronto-nasal sutures being connected to the rabbits facial skeleton.

MATERIALS AND METHODS

Animals

72 male New Zealand white rabbits, aged approximately four weeks were used. The animals were kept at $20^{\circ} - 21^{\circ} \text{ C}$ (40 % relative humidity) with light from 0600 to 1800 hours. Standard pellets and water were provided ad libitum. The rabbits were followed until age 5 months since, according to Engdahl (1972), a levelling off in their growth curve is shown at that age. Identical control groups were used in papers V and VI. Furthermore, 14 rabbits appear in more than one paper. An illustration of the rabbit cranial vault is presented in Fig. 1.

Bone markers

Since tantalum has the two properties needed, a high density to roentgen rays (atomic number 73) and a high inertness to body tissues, tantalum balls (99.9 % purity) of 0.5 and 0.8 mm in diameter (mass 1 and 4 mg, respectively) were selected as bone markers. Aberrations in marker dimension from perfect sphericity were less than $1 \mu\text{m}$ (Aronson 1976).

Balls were preferred over pins due to the very thin calvarial bone in the rabbit at age 4 weeks. Also, the radiation dose is reduced considerably by using balls, since the tip of a pin needs extremely standardized projections and high resolution film for identification (Aronson 1976).

Implantation instrument

The bone markers were introduced into the bone by a modified version (new release mechanism) of an instrument constructed by Aronson, Holst and Selvik (1974). A needle made of 18-10 Sandviken steel/2.5 % Mb alloy (Unimed S.A., Lausanne) was attached at the tip of the instrument; its inner diameter corresponding to the size of the bone marker used.

To facilitate manipulation of the instrument and to secure the implantation by minimizing the risk of uncontrolled penetration into the cranial bone, cylindrical stop-blocks were mounted on the needle, leaving respectively 0.5 and 0.75 mm of the tip exposed. This also made it readily pos-

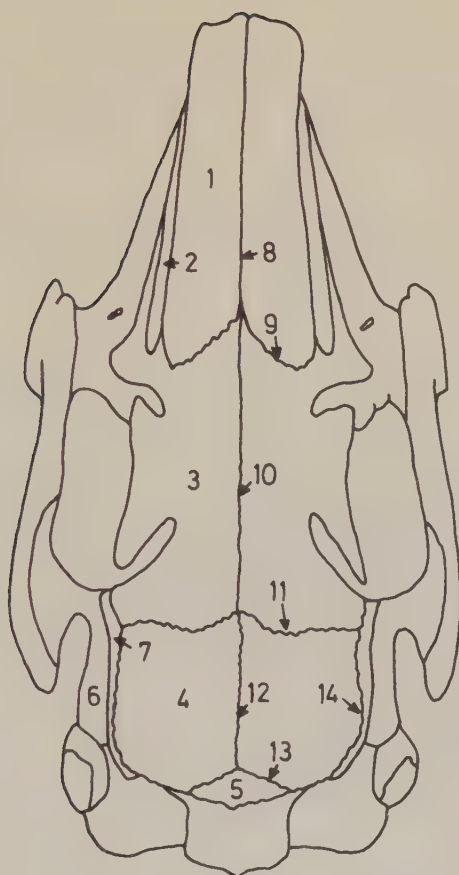


Fig. 1 Relevant anatomy of the cranial vault in rabbits.

Bones: 1. Nasal, 2. Premaxilla, 3. Frontal, 4. Parietal, 5. Interparietal and 6. Temporal.

7. Temporal line.

Sutures: 8. Internasal, 9. Frontonasal, 10. Interfrontal, 11. Coronal, 12. Interparietal, 13. Lambdoidal and 14. Temporal.

sible to visually inspect marker placement.

Immediately before insertion into the bone, the tantalum ball was placed into the tip of the needle. This was introduced into the bone and stabilized by rotating and forcing the bevelled needle tip into the bone surface, before pressing the trigger to release the spring of the inserting mechanism. Tibial implantations, though, were performed under fluoroscopic control by means of an angiographic needle. Tantalum balls (0.5 mm) were bilaterally inserted percutaneously in the proximal and distal epiphyses.

Bone marker positioning

The tantalum implants - at least two balls in each bone (called a segment); minimally three in kinematic calculations - were positioned in the nasal, frontal and parietal bones (Fig. 1) sufficiently far apart to ascertain the highest degree of precision. To estimate volume changes, bone markers were also implanted below the temporal line, making possible delineation of the major part of the cerebral hemispheres.

Two principles regulated the implants location (longitudinal growth):

1. The line connecting the markers in a rostral-caudal direction was to parallel the median plane.
2. The line connecting the bone implants on both sides of the sagittal suture was to be at right angles to this suture.

However, since relative positions of the balls changed during the observation period due to differences in magnitude of sutural (inter-bone) growth between the segments, minor intersegmental deviations from the original positions had to be accepted.

Bone marker requirements

Instability was registered by comparing distances as well as angles and rigid-body fitting (kinematic calculations) between implants in the same segment. A value exceeding the technical error (see below) implied that at least one marker was loose. The following criteria were used to standard-

ize the evaluation:

1. Only markers showing intrasegmental stability throughout the investigation were accepted.
2. The two most ideally placed markers on each side of a suture, according to the principles of implantation, were selected for longitudinal growth evaluations.

To reduce the influence of technical errors on growth rate values, the coronal suture as well as the sagittal suture, whose growth between successive measurements approached the technical error, were observed for minimally two- or three-week intervals, respectively.

Roentgen stereophotogrammetric equipment

To determine the geometry at radiography a calibration "cage" was used (Fig. 2). The cage consists of an upper and a lower 240 x 330 x 5 mm thermostable glass plate held 140 mm apart by parallel plexiglass sides. Each plate holds 9 radiopaque indicators (0.5 mm tantalum balls), their relative positions determined with high accuracy, which were recorded on the same film as the object.

A vacuum-packed plastic envelope enclosing an 18 x 24 cm mammography film together with an intensifying screen (gadoliniumoxysulphide, requiring lower radiation doses than envelope packed industrial film) was tightly pressed between the bottom plate of the calibration cage and a plane steel plate. Two simultaneously exposing roentgen tubes, with focal spots of 1.0 x 1.0 mm, were positioned with an approximate focus to focus distance of 0.4 m and a focus to film distance of 0.9 m.

Prior to exposure, a lead shield with a window at one end was placed on top of the cage to reduce scattered radiation to the film and to enable two stereo examinations on one film. During these examinations the reference indicators were covered. Use of a relatively narrow window prevented overlap of the stereo exposures.

Exposure data for a rabbit cranial vault examination were 48 - 51 kV and

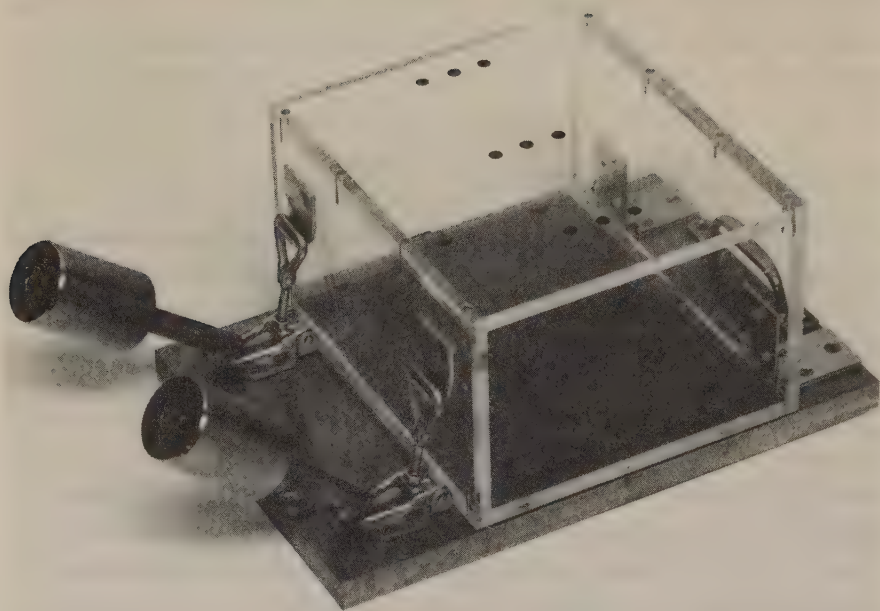


Fig. 2 The calibration cage construction used in the present investigation. Each plate holds 9 radiopaque indicators (0.5 mm tantalum balls). The triangles indicate fiducial marks, and the dots control points. The film is pressed between the bottom plate and the steel plate.

4 - 16 mAs, depending on age; but for recording the reference indicators and tibial markers, only 48 kV and 4 mAs were needed.

Exposure procedures

The exposure procedure was divided into two steps:

Step_I: The unanaesthetized rabbit was wrapped up in a cloth and placed in the calibration cage. The head (or tibial bone) was radiographed through the 75 x 80 mm window in the lead shield placed on top of the calibration cage.

Step_II: After carefully removing the rabbit and the lead shield, the previously exposed areas of the film were covered, and the cage indicators radiographed.

Sedation of the rabbits was quite infrequent and was only performed when adult rabbits were very uneasy and therefore hard to handle.

Film and computer analysis

The stereophotogrammetric analysis was done in three steps:

Step_1: The indicators of the bottom plate of the calibration cage, called fiducial marks following photogrammetric terminology, were used to correlate the film to the laboratory coordinate system defined by the cage.

Step_2: The indicators of the upper plate, called control points, were utilized to compute the positions of the two roentgen foci in the laboratory coordinate system.

Step_3: The intersection of the two rays through a radiopaque marker in the rabbit skull, called object point, was calculated by utilizing the computed projections of the object points on the plane of the fiducial marks (Step 1) and the just determined foci coordinates (Step 2), resulting in 3-D marker coordinates.

The 2-D film coordinates of the indicators of the calibration cage and the implants inserted in the calvarial bones (or tibial bone) were measured in a digitization instrument (Wild Autograph A8). Television display was

used on films with bad contrast. Calculations were performed by computer (Univac 1100/80). Spatial coordinates thus obtained were initially stored on punched cards together with the object identification number, and later stored in the computer system. The following computer programs were applied:

1. X-RAY for the calculation of implant 3-D coordinates from given 2-D coordinates of the markers in the calibration cage and measured film coordinates.
2. KINEMA for calculations of rigid-body motion between segmental implant configurations as well as implant translations in relation to a reference bone (point motion).
3. ROTATION for performing reorientation of the head.
4. GROWTH. This program calculates distances and distance changes from given 3-D coordinates of implants using the 3-D Pythagorean theorem.
5. MEDVOL for calculations of polyhedron volume and volume changes from a set of implant 3-D coordinates.
6. Plotting part of MEDVOL. For plotting the orthogonal projections of the implants on the coordinate planes; and also of implant positions at various investigations in relation to a common reference, using the program KINEMA.

Volumetric registrations (V, VI)

Volume was calculated from a polyhedron with corners at all markers excepting the nasal bone markers, constructed as the closest to convex polyhedron in a specific sense (Claesson et al. 1978) from the given marker configuration.

Kinematic calculations (IV)

Three stable bone markers, at least, constitute a rigid-body model (RBM) and thereby represent a segment (bone) in calculations of motion. The quality of rigid-body fitting was expressed as the root mean square distance (error) between segment markers in a rigid-body displacement compared to their actual movement. This mean error of rigid-body fitting (e_{rb}) includes both implants deviations from their original positions in the bone (assumed

rigid) and measurement errors.

In evaluations, one RBM was regarded as fixed. At each examination this segment was reoriented to its original position in the laboratory system by computer. Spatial motions between skeletal segments were described as rotations about the three cardinal axes, following classical kinematic principles for rigid-body movement. The axes were oriented as follows. The longitudinal axis is situated in the median plane directed antero-posteriorly. The transverse axis is in the horizontal plane, directed at a right angle to the longitudinal axis. The vertical axis is perpendicular to these.

The segmental translations presented are those of the center of gravity of the implanted markers, thus representing an approximate midpoint of the bone. Translations were given both as their components in the directions of the cardinal axes and as total translation, i.e. the distance calculated from these components using the 3-D Pythagorean theorem.

Technical accuracy

To test the accuracy of growth, volumetric and kinematic registrations, two exposures with different orientations were obtained of the same rabbit skulls.

Operative technique

The animals were anaesthetized by intramuscular neuroleptanalgesia (Hypnorm[®] vet.: Fluanizon and Phentanyl, 10 and 0.2 mg/ml, respectively; 0.6 ml/kg body weight). The head was shaved and the surgical area cleaned with alcoholic Chlorhexidin (5 mg/ml). A para-midline skin incision from the parietal to the nasal region was made. The skin and underlying tissues were reflected laterally and calvarial sutures identified (Fig. 1). Tantalum bone markers were implanted as described above. Care was taken to minimize periosteal manipulation so as not to influence bone production. After the implantation of bone markers the incision was closed with interrupted 4/0 Ethicon sutures and covered with Nobecutan[®] spray. Nebocetin[®] antibiotic powder (Neomycin 0.33 g, Bacitracin 25.000 IU) was sprinkled over the open wound in papers V and VI. The animals were left to recover on a warm

electric blanket.

Suturectomy (V)

Initially, 7 mm of periosteum on each side of the coronal suture was extirpated and the suture, including 5 mm of adjacently located parietal and frontal bone, was removed with a burr mounted on a dental drill. Bilateral coronal suturectomies included the adjacent part of the temporal bone and unilateral extirpations (left side), extended slightly across the midline. Extreme care was taken to avoid damaging the underlying dura. Bone wax was fixed to the bone edges and hemostasis secured. Zenkers modified solution (without glacial acetic acid) was applied to the dura with cotton swabs for one minute, avoiding the superior sagittal sinus, to retard bone regeneration (Anderson and Johnson 1956). After copious irrigation of the surgical field with sterile saline, the wound was treated as described above.

Temporary sutural immobilization (VI)

Small strips of periosteum (3 x 6 mm) were excised from the parietal and frontal bones parallel to the coronal suture, leaving the periosteum across the suture intact. A dental drill was used to roughen the exposed bone, after which a layer of isobutyl-2-cyanoacrylate adhesive (Bucrylat[®], Ethicon) was bilaterally applied across the coronal suture, to obtain sutural immobilization. The sagittal suture was carefully avoided. The glue was allowed to polymerize and the wound was subsequently closed as above.

After 15 or 42 days of sutural immobilization, the coronal suture area was re-exposed and linear craniectomy (= bilateral suturectomy) performed.

SEM procedure (II)

A diamond-edged wheel attached to a dental drill was used to remove the craniofacial bones (nasal, frontal, parietal) from each specimen, which were subsequently examined under a dissecting microscope. Only properly implanted tantalum balls, that is embedded in bone, were prepared for SEM.

Half of the bone specimens were immersed in 2.5 % glutaraldehyde in 0.2 M cacodylate buffer, pH = 7.2, the remainder were air-dried. Specimens were fixed for three hours (room temperature; RT), washed in cacodylate buffer (RT), dehydrated in graded series of alcohols and critical point dried using CO₂ as a transitional fluid.

The markers were localized roentgenographically, after which they were exposed for microscopic study by fracturing the bone. The specimens were mounted on SEM stubs, coated with gold/palladium in a vacuum sputter and examined in a Cambridge stereoscan Mark II A scanning electron microscope.

Macroscopic investigation (V, VI)

At the termination of the experimental period the degree of bone regeneration was controlled post-mortem under a dissecting microscope (Zeiss, Opton).

Using a protractor, dorsal radiographs were analyzed to determine whether snout deviation from the neurocranial midline was present. Any deviations were recorded to the nearest one-half of a degree in absolute figures.

Exposure dose measurements

To determine the radiation dose to the skull for each stereophotogrammetric exposure thermoluminescent dosimeters were attached to the top of the cranial vault on two rabbits. Dosimeters were also placed underneath the head to test the exit dose.

RESULTS

Technical aspects on RSA (I, III, IV)

The reconstruction of 3-D bone marker coordinates requires an exact knowledge of the geometry of the roentgen equipment used. Highly accurately determined calibration cage marker coordinates in combination with precise measuring instruments (constructed for cartography) minimize technical errors, and measures of quality were presented for the steps in the reconstruction procedure.

By using a similarity transformation instead of a central projection for image reconstruction, the time for measuring the stereo radiographs was shortened without a substantially lowered measuring accuracy. The presently used mammography film gave slightly lower precision than previously employed film/screen combinations. Nevertheless, errors in distance measurements were only 10 - 15 μm .

The effect of unfavorable marker positioning over a calvarial suture, not on line with a particular growth direction, was analyzed. An underestimation of growth rates is obtained which, however, will be slight.

The performed tests of accuracy in cranial vault growth measurements in rabbits are presented in Table I.

Bone reactions to tantalum markers (II)

To elucidate intramembranous bone reactions to tantalum markers and the histological basis for RSA-verified initial instability, a SEM study was undertaken at weeks 1, 2, 4 and 16 following implantation.

One week following tantalum bone markers (0.8 mm in diameter) implantation, the holes in the external cortical plate was clearly visible macroscopically. SEM disclosed a fibroblastic zone surrounding the implant, only random bone processes touching and adhering to its rather rough sur-

Table I. Accuracy of RSA measurements. Two stereo examinations were performed in two different positions of the rabbit skulls. The results of the exposures were thereafter compared according to the formula:

$$ME = \sqrt{\frac{(d_1 - d_2)^2}{(2 \times n)}}$$

where

ME = mean error

$d_1 - d_2$ = difference between measurements

n = number of measurements.

The denominator $2 \times n$ was used for volume calculations, since this only needs one examination. Axes: X = transversal, Y = longitudinal, Z = vertical.

GROWTH		21 μ m
KINEMA	rotation:	X 0.25°
		Y 0.43°
		Z 0.16°
	translation:	X 50 μ m
		Y 26 μ m
		Z 84 μ m
VOLUME		0.5 %

face. After two weeks, the implantation channels were covered by a thin film of bone and, microscopically, widely dispersed bone spicules were seen to contact the tantalum ball.

Four weeks following implantation the holes in the external cortical plate were hardly discernible. By SEM, frequent direct contacts between the marker surfaces and scattered bone bridges, so called osseointegration, were revealed.

Finally, after 16 weeks, the implantation channels were impossible to distinguish. The microscopic appearance resembled that at four weeks with the exception of a sparsity of soft tissues. The bone to implant attachment seemed solid, since fracturing did not ordinarily expose the marker. Surface comparisons of new and implanted markers revealed no signs of corrosion. No dissimilar reactions toward the implants between splanchno- and neurocranial bones were detected.

RSA-verified instability, although infrequent, was registered mainly in the nasal bones. In these cases, almost complete marker penetration through the calvarial bone was observed.

Post-implantation cranial vault growth (III, IV)

Bone marker instability, defined as intrasegmental marker distance variations exceeding twice the technical error, occurred infrequently and was mainly restricted to the nasal bones. Complete marker loss was rare and probably due to the implantation procedure.

The maximally observed exposure dose for a stereo examination was 0.6 mGy (51 kV, 16 mAs).

Translatory growth at the frontonasal suture, directed about 45° downwards relative to the frontal bones, exhibited the highest rates observed during the investigated period. A successive decrease between age 4 to 21 weeks from about 260 to 61 $\mu\text{m/day}$, with intermittent differences between right and left sides, was observed. Furthermore, individual rate fluctu-

ations, with periods of subnormal growth followed by compensatory rebounds were registered. When studying two marker pairs across the same suture side, minor rate variations could be detected. This was not noted for the coronal suture. Also, the nasal bones rotated downwards (8°) relative to the frontal bones.

At the coronal suture, distance changes between markers were fairly well represented by longitudinal and total translations. Here, growth rates declined from 53 to 13 $\mu\text{m}/\text{day}$. Viewed as a group, differences between sides as well as growth fluctuations were not as marked here, but on an individual basis, significant variations were shown. During the investigated interval the frontal bones rotated rostrally upwards (12°), outwards (3°) as well as laterally downwards (5°) in relation to the parietal bones.

Sagittal sutural growth was exceedingly small compared to other sutures studied, even though more constant in nature. Rates for neurocranial parts, generally less than 11 $\mu\text{m}/\text{day}$, occasionally exhibited negative growth values. The frontal bones demonstrated a laterally downwards rotation (2°), while that of parietal bones was laterally upwards (3°) relative to their contralaterals.

Interestingly, regular weight controls were not found adequate in determining individual animal health as suggested by frontonasal sutural growth (VII).

Craniofacial (nasal markers included; not previously presented) and calvarial volumes increased 86 % ($n = 12$) and 17 % ($n = 11$), respectively, during the investigation period. This is illustrated in Fig. 3, together with craniofacial plottings of initial and final polyhedral configurations for one rabbit.

Neurocranial suturectomy (V)

Generally, experimental groups paralleled controls frontonasal sutural

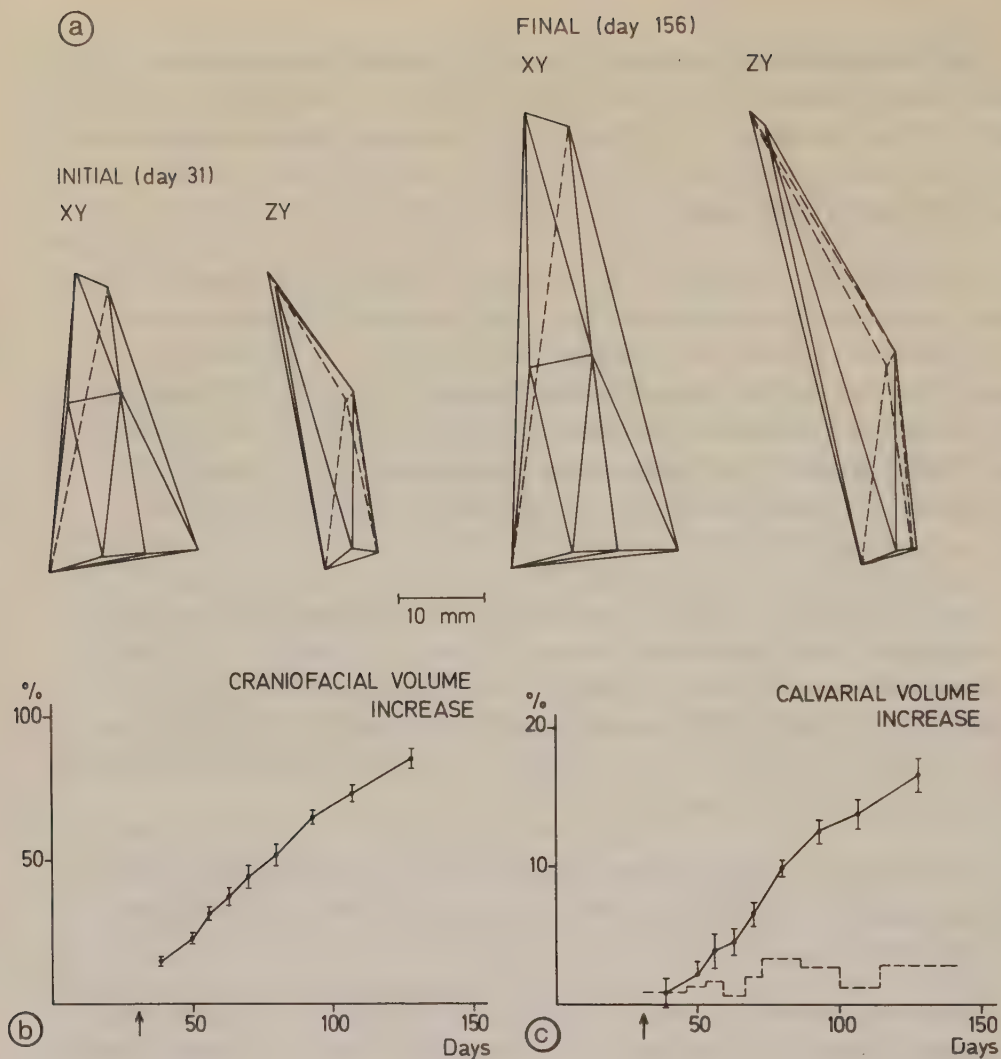


Fig. 3 Registered cranial volumes. a/ Initial and final configurations, constructed of bone markers in the nasal, frontal, parietal and temporal bones, in one rabbit as projected on the frontal (XY) and sagittal (ZY) planes. The configurations are oriented in as identical positions as possible using an optimization principle. b/ Craniofacial volume increase. Increase of polyhedral volume as related to initial volume. Arrow marks start of investigation. c/ Calvarial volume increase (nasal bone markers excluded). Rate change for each interval included (---).

growth rates, showing only minor deviations. Bilateral coronal suturectomy resulted in slightly increased growth rates initially and terminally, while the contrary applied to unilateral extirpation. Also, the growth rate fall tapered off during the last part of the investigation, most notable in bilaterally suturectomized animals. This was not registered in controls.

Bilateral coronal suturectomy, especially initially, significantly enhanced frontal and parietal bone separation compared to unilaterally extirpated and control animals. Nevertheless, unilaterally suturectomized rabbits showed increased initial marker separation at the extirpated side. Also, both experimental groups demonstrated increased growth rates during the final period. Furthermore, cranial vault width increased in bilaterally extirpated rabbits.

Coronal suturectomy only slightly affected sagittal sutural growth. Only a reduced initial interfrontal sutural growth rate was registered following unilateral extirpation.

Neurocranial polyhedron volume was observed to increase continuously during the whole observation period in controls. No terminal levelling off, coupled to the diminished longitudinal craniofacial growth, could be traced. Bilateral extirpation produced a significantly increased near-parallel volumetric expansion relative to controls, finally reaching a plateau, while unilateral suturectomy, after an initially elevated increase, finally conformed to controls.

A slightly pronounced increase of snout deviation was noted following unilateral suturectomy relative to bilaterally extirpated and control animals.

Restricted periods of sutural immobilization (VI)

The frontonasal sutural growth rates of experimental animals paralleled controls during the major part of the investigation. However, release of coronal sutural growth restriction resulted in periods of reduced growth rates in both groups, which shortly returned to control rates.

The immobilization of the coronal suture proved effective during both intervals (2 or 6 weeks). Surgical interruption of growth restriction resulted in significantly accelerated separation of the frontal and parietal bone margins, especially after early linear craniectomy. During the remainder of the studied period, growth rates exceeded controls considerably in both groups.

As for the sagittal suture, only the interfrontal suture significantly deviated from controls by increased initial and final rates.

Calvarial volume remained similar to controls during short and prolonged immobilization. Only early craniectomy resulted in significantly increased volumes, which stayed supranormal during the rest of the investigation, while volume after late release of growth restriction only exceeded controls terminally.

Macroscopically, a rostrally upward displacement of the parietal bones relative to the frontal bones was attained in animals subjected to prolonged immobilization. This was not observed following short period growth restriction. Lateral snout deviation was comparable to controls.

The bilaterally implanted tibial epiphyseal markers demonstrated successively decreasing rates of marker separation, which were especially marked following early craniectomy. Although the slight growth rate depression after late surgical intervention was not significant, a subsequent period of compensatorily increased growth was registered. Also, tibial tantalum bone marker implantation generally seemed to reduce growth slightly.

Comparison of membranous and chondral longitudinal bone growth (VII)

With the exception of a short temporary growth reduction due to decreased nutritional intake, craniofacial growth conformed to rabbits as described above (III). Tibial growth, left and right sides closely following each other, demonstrated a growth curve nearly parallelling the fronto-nasal suture. This was especially evident during the period of nutritional

deficiency.

In one animal, presented individually, even growth at the coronal suture was shown to react to reduced nutrition, and consequently exhibited a diminished marker separation during this period. This was even more evident in another animal, excluded from the rest of this study, which was unvoluntarily exposed to prolonged nutritional shortage (Fig. 4). Early observations revealed this rabbit grew more slowly than other experimental rabbits. Different steps were undertaken to find the cause of this. During week 9, we observed the rabbit being prevented from eating properly by its cage mate, hence they were separated. Tibial and frontonasal sutural growth, closely adhering to each other, were observed to be markedly decreased during the initially investigated weeks, contrary to the coronal suture. The separation during week 9 resulted in a pronounced rebound in growth, also pertaining to the coronal suture. Excepting the coronal suture, growth was not fully compensated during the investigated period, as compared to remaining animals.

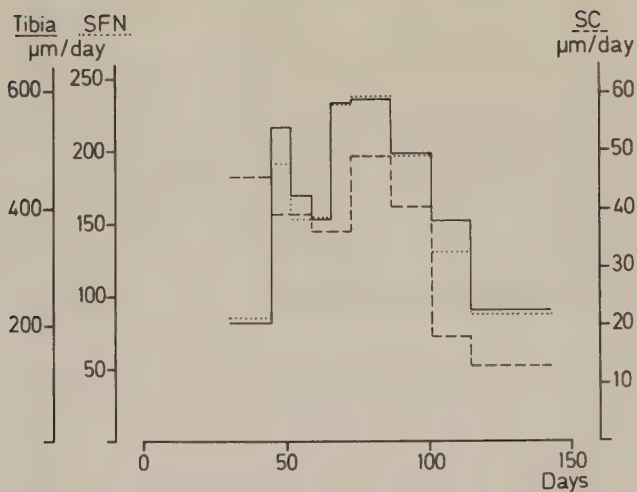


Fig. 4 Illustration of tibial and antero-posteriorly growing sutures growth rates for one rabbit, which was temporarily prevented from eating properly. The tibia and the frontonasal suture demonstrated a parallelling growth pattern. Note that the pronounced rebound after resumption of eating also pertains to the coronal suture. SFN = frontonasal suture (---), SC = coronal suture (---).

GENERAL DISCUSSION

Methodological aspects

Roentgen stereophotogrammetry is a technique of obtaining 3-D coordinates from radiographs, thereby geometrically characterizing an object. However, an accurate calibration of the radiographic equipment is required. Equipment calibration was performed simultaneously with that of a 3-D test object, which subsequently demonstrated that a high accuracy (about 10 μm) can be achieved. Thus, usage of standard roentgen equipment combined with a photogrammetric instrument (Wild A8), gave highly accurate image reconstructions. Hereby, 3-D distance, the primary measure in the present investigation, was determined with an accuracy of 10 - 15 μm .

Fundamental to RSA are well-defined landmarks. Since the rabbit skull lacks any such distinct marks tantalum balls were implanted. Björk (1963) introduced the use of tantalum implants into the human craniofacial skeleton to improve reorientation in roentgen cephalometry. Recently, Grundschober et al. (1980) reported osseointegration (= a direct bone-to-implant contact) for endosseously implanted dental tantalum screws, which had been implanted in man for 8 - 12 years. Osseointegration can be explained by several factors, among which are the material used, its design, healthy bone tissue as well as stability (Albrektsson et al. 1981). As to surrounding tissues, Aronson and Jonsson (1976) found no or only slight fibrosis at the implantation site, and usually only a mild inflammatory soft tissue reaction, using the same implantation technique as we have used. We found an immediate fibrocytic invasion producing a fibrous network which rapidly adhered, although fragilly, to the tantalum balls surface. Thereafter, bone healing progressed towards a stable osseointegration, with similar responses in splanchno- and neurocranial bones. The clinically observed initial marker instability (e.g. Rune et al. 1979, 1981, Bylander et al. 1981) may thus be due to the healing process showing initial irregular fibrous adhesions with only sparse bony support, but may also result from intrinsic bone elasticity.

The inertness of tantalum to body tissues has previously been confirmed by a multitude of investigators, e.g. Burke (1940), Samuels et al. (1966), Nadel et al. (1968), McFadden (1969), Grundschober et al. (1980), and was also corroborated in this study. Nevertheless, a case of chronic urticaria from tantalum staples was recently reported (Werman and Rietschel 1981), which calls for awareness of eventual side-effects. However, taking into account tantalums wide usage in many different fields of medicine, and the abundance of investigations concerning its biological effects, it may be concluded that tantalum is very suitable for use as a bone marker and an excellent alternative to e.g. titanium in skeletal surgery.

Cranial vault growth

Cranial growth proceeds by individual bone changes in size, shape and spatial position. The sutures are arranged so as to permit increases in cranial length, width and height. Thus, neurocranial bones, separated by the volumetric expansion of the enclosed neural mass, obtain compensatory bone deposition at their margins. It is therefore reasonable to suppose that changes in the brain case reflect changes in brain size.

By RSA it was possible to demonstrate that the cranial vault was still expanding at age 5 months, when rabbit body growth is virtually completed (Engdahl 1972). Also, spatial rearrangements at calvarial sutures occurred up to maturity, implying that shaping of the cranial vault continues even when brain growth is known to be minimal (Harel et al. 1972).

Concerning longitudinal growth at transverse sutures, the frontonasal suture showed higher growth rates compared to those of most investigators (Selman and Sarnat 1955, Erickson and Ogilvie 1958, Hong et al. 1968, Roskjaer 1977, Koskinen-Moffett et al. 1981), while Babler et al. (1982a, b) reported an initial marker separation exceeding ours. As for the coronal suture, lesser differences in growth rates have been registered. Koskinen-Moffett et al. (1981) obtained values similar to ours, while those of Erickson and Ogilvie (1958) and Hong et al. (1968) were significantly lower. Even here the University of Virginia group (Persson et al. 1979,

Persing et al. 1981, Babler et al. 1982a, b) registered initial rates markedly exceeding ours.

This may be attributed to a multitude of factors e.g. the applied method (roentgen cephalometry or vital staining usually utilized), its accuracy, sex or strain differences, varying animal care (nutrition, litter size), time and extent of surgical trauma, postoperative health decline (see below). To optimize growth representation, several steps were undertaken, among which are the usage of only male rabbits from a well-controlled rabbit strain and strict rules for animal care. The implantation procedure, though, has been reported to only slightly affect long bone growth (Aronson and Hansson 1976), hence it is hardly likely to greatly affect the passive neurocranial growth. Interestingly, weight was found inadequate in determining individual animal health, which was also observed by Hansson (1967).

Furthermore, it was demonstrated that the positioning of bone markers affect growth registrations. Both an oblique location relative to the growth direction and omittance to consider bone rotations inevitably leads to incorrect measures. Nevertheless, a complex growth pattern may occur in individual cases, rendering optimal determination of growth very difficult.

As to kinematic analysis of frontonasal sutural growth, this was not parallel to the nasal bone, but directed inferiorly (forwards-downwards) in relation to this bone. However, the frontal bone rotated upwards at the coronal suture (12°) relative to the parietal bone. Also, in a coronal section, the opposite but in magnitude equally large rotations were found between the latter bones. Thus, torsional forces are developed in the coronal suture supporting earlier theories that the brain does not expand uniformly in all directions.

The high accuracy of RSA enabled detection of a continuous growth period even for the sagittal suture, which remained open throughout the investigation. This contradicts Hoyte (1971) who, using vital staining, reported

terminated neurocranial sagittal growth after about 40 days. This was probably due to this methods low sensitivity.

In individual sutures, it was possible to further characterize sutural growth. Ipsilateral variations in growth across a suture (the frontonasal suture) as well occasional negative growth values (the sagittal suture) indicate that elasticity of bone or sutural tissue and bone resorption are integral parts of sutural growth. Also, cranial vault bone separation is not a pure translational movement, but rather an oscillatory separation. This was further corroborated by kinematic analysis. Also, left-right differences (Moss 1954, Hong et al. 1968, Aaron 1973, Koskinen 1977, Sarinat and Selman 1977) and short-period fluctuations in growth rate (Koskinen 1977) were biometrically confirmed. Nevertheless, only very slight craniofacial asymmetry was observed. Thus, calvarial growth is part of a well-functioning inter-relating cranial growth process.

The predominance of facial skeletal growth during the period studied as well as a dominating coronal to frontonasal sutural rotation about the transverse axis (showing a final quotient of 1.5) signify an alteration of skull form from klinorhynchal (facial skeleton lies subcerebrally) to orthocranial (facial skeleton precerebrally), as suggested by several investigators e.g. Baer (1954), Moss (1958b), Pucciarelli (1981), Engström et al. (1982). Interestingly, both frontonasal and coronal sutures showed a decrease to one-fourth of the initial values despite different functional matrices (Moss and Salentijn 1969). Additionally, tibial growth also demonstrated a fall to one-fourth of initial values.

Following a brief period of nutritional deficiency a pronounced fall in growth rates were registered for the frontonasal suture and tibia. Shortly, though, a compensatory catch-up could be seen, which agrees with other investigations (review: Himes 1978). The separately described rabbits (p. 33) point to the very intimate relation between the tibia (long bones) and frontonasal suture (facial skeleton) during this interval. The coronal suture, though, exhibited growth less affected by external influences. However, pronounced reductions as well as compensatory rebounds were registered

after prolonged nutritional shortage.

The brain is known to have a high priority for growth and a relative resistance to undernutrition. Nutritional deficiency has repeatedly been shown to only slightly affect brain growth, e.g. Adlard and Dobbing (1972), McAnulty and Dickerson (1974), resulting in less delay in neurocranial development than splanchnocranial (Riesenfeld 1967, 1973, Williams and Hughes 1978, Pucciarelli 1980, 1981). Nevertheless, assuming that calvarial changes reflect changes in brain size, influences on cerebral volume were demonstrated to occur rapidly.

Growth after trauma to the coronal suture

What is the reaction of the calvarium to removal of a suture? Various facial and neurocranial sutures have been experimentally extirpated in different species, usually without any effects on longitudinal growth or macroscopical morphology (Levine 1948, Moss 1954, Selman and Sarnat 1957, Watanabe et al. 1957, Sarnat 1958, Lynch and Peil 1966, Stenström and Thilander 1967, Engdahl 1972, Smith and McKeown 1974). However, adjacent skeletal and soft tissue connections to the sutural area have frequently been neglected, and inaccurate measuring procedures applied.

To minimize factors of external interference on the experimental area, the coronal suture was selected in the present study. This suture, when growing, provides the advantage of being influenced mainly by one functional matrix, the expanding neural mass (Moss and Salentijn 1969). Furthermore, no restricting tissues seem to exist.

After bilateral coronal suturectomy, a significantly accelerated separation of the frontal and parietal bones and an increased cranial vault width as well as a dramatic calvarial volumetric expansion was observed. Unilateral extirpation, on the other hand, initially showed increased bone separation on the extirpated side combined with elevated volumetric expansion, and slightly more pronounced terminal nasal deflection. Presumably, these results express a liberation of biomechanical stresses due to neural mass growth, implying that the sutural tissue proper is a restrain-

ing and also modulating component during normal growth. Furthermore, this supports the passive nature of cranial vault growth.

Similar conclusions have recently been drawn by the previously mentioned group at the University of Virginia, USA (Persson et al. 1979, Persing et al. 1981, Babler et al. 1982a), using roentgen cephalometry in combination with measurements between dental amalgam implants. Contrary to our results, though, they found reduced growth at the frontonasal suture after bilateral extirpation. Changed growth at this suture might be due to spatial rearrangements of cranial bones (Persson et al. 1979, Babler et al. 1982a, b) as the later maturing facial structures may offer a greater capacity for calvarial adjustments (Persson et al. 1979). However, this divergence may also be explained by variations in animal health.

The sagittal suture, previously not studied during these circumstances, demonstrated minor changes. The interfrontal suture, showing an initial growth rate fall after unilateral suturectomy, thus demonstrated that even slow-growing sutures are sensitive compensatory sites.

Experimental craniosynostosis (strictly speaking craniosynostosis refers to the process of premature sutural bony fusion) has been more or less successfully produced by many means. Recently, cyanoacrylate adhesive was introduced, offering the advantages of an immediate immobilization of the sutural area as well as skull base deformations similar to human cranio-stenosis (the aberrant skull shape resulting from sutural synostosis). Surgeons now prefer early intervention, even though premature fusion may not be detected for some years. Since the time of onset of sutural growth restraint is not known clinically, the compensatory reactions at other sutures as well as the time lapse before the début of obvious clinical symptoms remain obscure.

In this study, two different durations of growth restriction were investigated. The cyanoacrylic bridge remained effective even during the 42 day interval, possibly developing an ectocranial bone bridge during this period (Persson et al. 1979, Foley and Kokich 1980, Nappen and Kokich 1983).

During the period of antero-posterior growth restriction calvarial volume and frontonasal sutural growth remained similar to controls. However, Babler et al. (1982b) registered a growth decrease at this suture. On the other hand, a substantial compensatorily increased interfrontal sutural growth was shown in both groups. The latter observation contradicts a radionuclide investigation by Gates and Dore (1975), who found growth at non-fused sutures to be normal or only slightly accelerated.

Linear craniectomy caused both a dramatic bone separation at the coronal suture as well as a volumetric expansion exceeding controls. Even late craniectomy gave markedly increased growth rates. In both groups the supra-normal growth finally implied an overcompensation. Frontonasal sutural growth then decreased in both groups, which for animals subjected to early craniectomy was not compensated for during the investigated period. As previously discussed, this fall in growth may probably be attributed to a temporary health decline due to surgery. Consequently, frontonasal sutural growth is not an accurate determinant of cranial growth relations, but rather reflects somatic growth conditions.

However, the sagittal suture again showed abilities to compensate for changes in cranial vault growth. The interfrontal sutural separation normalized rapidly while discontinued growth at the interparietal suture was registered in some animals following late craniectomy. Consequently, increased growth at one suture is accompanied by reduced growth at another, hereby anew supporting the passive nature of growth at the calvarial sutures.

Speculatively, the latter two investigations point to the importance of careful planning of surgical manipulation of neurocranial sutures during infancy, i.e. the positioning, time, and extent of surgery, to obtain optimal cosmetic and functional results as well as to avoid disturbances of normally developing areas. Furthermore, the highly accurate RSA has been found suitable for experimental investigations on cranial growth and hence enables studies on many types of cranial aberrations.

GENERAL SUMMARY

Cranial vault growth in New Zealand white rabbits was studied by roentgen stereophotogrammetric analysis (RSA). This method is based on measurements of radiopaque bone marker images on radiographs. Two roentgen tubes simultaneously expose the object, which is placed in a calibration cage. The cage, holding indicators in two planes of known internal positions, defines the laboratory coordinate system. The 2-D image coordinates are obtained by means of a highly accurate cartographic instrument (Wild Auto-graph A8). By computer, 3-D object coordinates are subsequently calculated. Several computer programs are available for various analyses of growth processes. The construction, calibration of the system as well as its technical accuracy has been discussed in detail.

Bone reactions to the tantalum markers, used as landmarks, were studied by scanning electron microscopy. Initially, a fibrous network adhered to the markers surfaces, whereafter bone healing progressed towards osseointegration, facial and calvarial bones responding similarly. Hereby the bioinertness of tantalum was confirmed.

The highly accurate RSA enabled further characterization of cranial vault sutural growth. Ipsilateral variations in growth across a suture and occasional negative growth rate values indicated that bone separation is an oscillatory movement, bone resorption thus being an integral part of sutural growth. This also applies to the left-right differences and short period fluctuations registered. Furthermore, spatial bone rearrangements were shown to occur up to maturity, resulting in e.g. torsional forces in the coronal suture.

Tibial growth closely paralleled frontonasal sutural growth, which consequently primarily disclosed external disturbances on cranial growth. The coronal suture was seen to react to nutritional deficiency, although to a lesser degree than the previously mentioned areas.

Coronal suturectomy revealed this neurocranial suture to be a restrain-

ing, passively growing component in normal growth. The sagittal suture demonstrated the calvarial compensatory ability.

Various periods of antero-posterior growth restriction revealed substantial compensatory interfrontal sutural growth. The release of growth restriction by linear craniectomy, following both short and prolonged immobilization, resulted in dramatically increased frontal and parietal bone separation, ultimately showing an overcompensation. Additionally, calvarial volume increased whereas lateral growth diminished or normalized.

In conclusion, the cranial vault sutures in rabbits seem to partake in a delicate but well-functioning holistic growth process.

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A ROENTGEN STEREOPHOTOGRAMMETRIC SYSTEM.

CONSTRUCTION, CALIBRATION AND TECHNICAL ACCURACY.

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INTRODUCTION

Roentgen stereophotogrammetry is the science of obtaining reliable three-dimensional (3-D) measurements from radiographs in order to determine, primarily, geometric characteristics of an object.

Already in 1897, two years after Roentgen's discovery of "x-rays", Davidson and Hedley as well as Payne described principles for 3-D localization from radiographs. Since then, many attempts to spatially reconstruct objects have been made (reviews: Hasselwander 1954, Köhnle 1967, Hallert 1970). Nevertheless, no systematic use of roentgen stereophotogrammetric analysis (RSA) seems to have occurred until the 1970's, when several techniques were developed in various centers. However, RSA seems to have been most comprehensively used in Lund, Sweden (review: Selvik, 1982).

Usually, distinct skeletal landmarks permitting accurate identification are missing in the skeleton, wherefore metallic markers are implanted to define the measurement points. In Lund, tantalum balls, 0.5 or 0.8 mm in diameter, were selected. Tantalum has the two properties needed of an implant metal, high inertness to body tissues and a high absorption of roentgen rays (atomic no. 73).

In general, a reconstruction of the marker coordinates at the moment of roentgen exposure requires that the geometry of the roentgen equipment is known. This enables a mathematical reconstruction of the bundles of roentgen rays. Since standard radiographic equipment is utilized, its geometry must be ascertained by some means of calibration equipment. For this purpose, radiolucent "cages" with radiopaque markers of known 3-D internal positions are employed. A particular calibration cage that has proven suitable for high-accuracy measurements particularly of distances and distance changes will be described. Furthermore, the calibration of the calibration equipment itself is of fundamental importance. The technical accuracy (accuracy = the closeness to truth of measurements) for various

procedures comprised in calibration and utilization will be analyzed. High accuracy requires high precision, i.e. a low dispersion around a mean value, and aspects on the precision in the measurement procedures will therefore be presented.

TECHNICAL DESCRIPTION

Construction of a calibration cage

The calibration cage was constructed from two 5 mm thick 240 x 230 mm rectangular glass plates. The two plates were fixed parallelly by means of two 240 x 135 x 15 mm plexiglass plates. These were glued with Araldite® (Ciba) to the long sides of the glass plates, positioning these at a distance of 135 mm apart. Thus a "cage" with two openings at its short sides was obtained. Nine tantalum balls (0.5 mm in diameter) were cemented on the inside of each glass plate using Super-Epoxy® (AB Plastic-Padding, Gothenburg, Sweden), a transparent epoxy glue.

2-D calibration of the cage

Prior to cage assembly, the relative positions of the indicators were determined in a photogrammetric instrument. For such, a Wild Autograph A8 or a Wild Stereocomparator StK-1 (Wild Heerbrugg AG, Heerbrugg, Switzerland) was used. The measured values were presented in a rectangular coordinate system with its origin in one short side marker and the y-axis defined as the longitudinal axis of the plate. To illustrate this 2-D calibration, the registered coordinates (Wild A8; the means of five settings of the instruments measuring mark) from two separate measurements of the lower plate are presented in Fig. 1. During measurement each plate was fixed in the instruments film-holder, whereafter the 2-D positions of the nine markers were measured. The root mean square values of the differences between the markers position were 10 and 7 μm (values obtained by congruency, rigid-body, fitting of the two coordinate sets using a least squares method; computer program KINEMA; see below) for the plate

in Fig. 1 and the upper plate, respectively. Thus, the precision in determining the marker position in one plane was close to $5\text{ }\mu\text{m}$. However, the instrument measuring accuracy was near $10\text{ }\mu\text{m}$ due to inexact linkage mechanism, resulting in a total coordinate accuracy approximating this value. For the Wild StK-1, whose instrument accuracy was close to $1\text{ }\mu\text{m}$, a measuring precision of $5\text{ }\mu\text{m}$ represents the accuracy of the procedure.

The tantalum ball markers were fixed to the glass plate with glue during the measuring procedure, leaving the upper poles of the spheres uncovered. Thereafter, they were covered in a thick coat of glue.

Cage marker function

The lower plate markers, called fiducial marks (FM), are utilized to transform the film coordinates to the 2-D laboratory coordinate system determined by these markers. The roentgen film is placed directly under the FM plate (Fig. 2). The indicators of the upper plate, called control points (CP), are used for determining the positions of the two roentgen foci in the above-mentioned laboratory coordinate system.

The mathematical procedures for using these markers to reconstruct the 3-D object coordinates have been extensively accounted for by Selvik (1974). In brief, the first step is to transform all measured film coordinates, both of FM:s, CP:s and object markers to the coordinate system defined by the lower plate. Two different types of transformations can be used - a central projective transformation determined by the images of four markers provided three are not on a line, or a similarity transformation assuming parallellicity between the film and FM plane, which requires only two markers. After this initial calculation (utilizing the FM:s) lines are constructed between the CP:s and their transformed film images. The point in space which has the least squared sum of distances to these lines is an optimal fit position of the roentgen focus. However, for this to be possible the CP marker coordinates must have been determined in the same 3-D coordinate system as the FM markers, i.e. the position of the upper plate in relation to the lower one must be settled.

3-D calibration of the cage

A more difficult task than determining the relative positions of markers in a plane is the assessment of 3-D positions of the two sets of markers in relation to each other. Each marker set has six degrees of freedom in conformity to the rigid-body concept, i.e. six parameters are required to define its position. These parameters can preferably be chosen as the translations along and rotations about the coordinate axes. In the present application the coordinate system was defined as follows. The y-axis is the longitudinal axis of the cage; the x-axis is perpendicular to the y-axis and lying in the plane of the fiducial marks, while the z-axis is perpendicular to this plane and pointing towards the CP plane (Fig. 2).

In the coordinate system thus defined, being the laboratory coordinate system to which all measurements will be referred, the positions of control points are to be determined. This is achieved by the following means.

1. The rotational degrees of freedom: By cutting both of the two plexiglass side-plates, constituting the cages sides, in one operation they obtain equal and uniform height. Thus, when assembling the cage, relative rotations between the plates about the x- and y-axis are impossible. However, a rotation about the z-axis is likely to occur when gluing the cage. An indirect method for computing the rotation about the z-axis was developed (Selvik, 1974), which, for larger test cages, gave standard deviations in determining the rotation angle of about $1 \cdot 10^{-3}$ degrees. In principle, for correctly given CP-coordinates, the sum of the squared distances between a focus and the lines through the CP:s and corresponding images will be minimal. For two cages of the present size, a rotation of 0.159° and 0.284° was registered. These values are the means of six determinations, the standard deviation for a single measurement amounting to 0.003° and 0.006° , respectively.

2. The translational degrees of freedom: The position in height (z-axis translation) of the CP:s is determined by the height of the plexi-

glass side pieces in combination with the glue thickness. Caliper measurements of the inter-plate distance have shown the glue layer thickness to be negligible.

The two plates are parallel to each other due to the fabrication of the side pieces as described above, but might be arbitrarily displaced along the xy-plane during gluing. Consider, for instance, a translation along the y-axis. If the given y-coordinates of the upper plate are too high, the calculated positions of the foci will obtain too high y-values (Fig. 3). Correspondingly, object y-coordinates will be too high, the errors varying linearly with the height (z-coordinate) from the lower plate. This deformation of model coordinates (the coordinates calculated for an object) can be used to determine the error in the given position of control points.

As demonstrated by Selvik (1974) but also evident from Fig. 3, an error d_y in y-coordinate of CP:s (height Z_{CP}) leads to an error Δy_P for an object point P at height Z_P which is

$$\Delta y_P = \frac{Z_P}{Z_{CP}} d_y \quad (1)$$

Thus, in this case, by radiographing a 3-D test object holding markers in two planes (Fig. 4), the upper plane markers will be assigned too high y-coordinate in relation to those in the lower plane. Consequently, this error will be

$$\Delta y_h = \frac{Z_h}{Z_{CP}} d_y \quad (2)$$

when Z_h is the height for the object. If the test object is rotated 180° about the z-axis, the model coordinates of the upper plane will be lower by the same amount (2), in a coordinate system fixed to the lower plane of the test object. The error of CP coordinates d_y can be calculated from the

difference of Δy in model coordinates between these two positions, as $\Delta y = 2\Delta y_h$.

Thus,

$$d_y = \frac{1}{2} \cdot \frac{Z_{CP}}{Z_h} \Delta y \quad (3)$$

Hereby, the required correction to given CP coordinates will be $-d_y$.

The difference Δy can either be calculated directly by comparing coordinate values between the positions in a coordinate system defined by three markers in the lower plane, or by kinematic analysis (see below), where Δy is the translation between the marker planes modelled as rigid bodies. This will be illustrated by an example (first calibration of the cage with lower plate in Fig. 1):

The distance between the glass plates of the calibration cage is 135 mm, with the tantalum markers (diameter of 0.5 mm) adhering to their inner-sides. Thus, $Z_{CP} = 134.5$ mm. The test object height $Z_h = 80$ mm. By radiographing the 3-D test object in the two positions mentioned, calculating model coordinates and subsequently the relative translation of the upper plate, the difference was found to be 0.452 mm. Thus, the required correction to given CP coordinates will be

$$-d_y = -\frac{1}{2} \cdot \frac{134.5}{80} \cdot 0.452 \text{ mm} = -0.380 \text{ mm}.$$

Of course, the same procedure applies to the x-direction, which correction is calculated from simultaneously obtained x-translations.

By repeating the complete calibration procedure and comparing the adjustments obtained, the precision was found to be excellent for y-values but somewhat lower for x-values, which might be explained by a shorter x-distance and slight flexibility of the cage. Thus, two separately determined x-corrections were 0.497 and 0.461 mm, respectively, whereas the y-corrections were -0.380 and -0.376 mm (example as above).

RESULTS

Film evaluation

Three film-intensifying screen combinations have been used for various applications: Agfa-Gevaert D7 Structurix envelope packed film (fine grain film; no screen), Du Pont standard film with Siemens Rubin Super double screens and vacuum packed mammography film with a single gadoliniumoxysulphide screen; all sized 18 x 24 cm. The film enclosed in paper or plastic envelopes was tightly pressed between the lower plate of the calibration cage and a highly polished plane steel plate to minimize film unflatness.

Film measuring is performed using a Wild Autograph A8. The film is pressed between the two glass plates of a film holder. The setting mark is a black circular dot with a diameter of 70 μm . The setting precision has been found to be 6.2 μm and 6.5 μm (D7), 5.5 μm and 5.6 μm (standard film/Rubin), 4.2 μm and 5.0 μm (mammography/gadoliniumoxysulphide) for x- and y-directions, respectively.

The first step in the reconstruction of the object is the transformation from film to the laboratory coordinates of the FM:s, which can be performed using either a central projection or a similarity transformation, as mentioned above. The mean error of fitting of measured film coordinates to the given cage coordinates was $15 \pm 4 \mu\text{m}$ (9 FM:s) and $64 \pm 9 \mu\text{m}$ (4 FM:s) using identical measurements with projective or similarity transformation, respectively.

The second step is the calculation of foci coordinates. The root mean square distance between a calculated focus and lines determining this focus was $0.282 \pm 0.060 \text{ mm}$ and $0.238 \pm 0.045 \text{ mm}$ for central projection (9 CP:s) or similarity transformation (4 CP:s), respectively (in all four cases the means $\pm$ SD of 12 focal determinations).

The third step, finally, is the intersectioning of corresponding rays between the foci and transformed image points. Here, a suitable measure of quality is the shortest distance between these two rays as they do not intersect but only pass each other at a distance. Typical values for this error vector lie around 10 μm , with a dispersion (SD) also of approximately 10 μm .

3-D calibration of a test object

By radiographing a 3-D test object (Fig. 4) in two positions, rotated 180° to each other about the z-axis, an error in model coordinates due to incorrect positional assignment of the CP:s can be annulled. This is accomplished by taking the mean from the two evaluations as the object coordinates. However, the z-positions of the object markers are determined with lower precision as will be discussed below. For this reason the test object is radiographed separately lying on its side to determine the z-coordinates. By calibrating the test object twice in this way, it was found that the root mean square value of the 3-D distances between the 44 markers when comparing the two evaluations was 11 μm . As no systematic error is present in this calibration, it can be concluded that a precision of 8 μm ($= \frac{11}{\sqrt{2}} \mu\text{m}$) is an expression for the calibration accuracy of the test object. The mean error of 11 μm corresponds to an error of 6 μm for each coordinate assuming equal precision in all three dimensions.

Accuracy of distance measurements

Tantalum balls, 0.5 mm in diameter, were glued to two glass plates, forming nine pairs; the distance between each marker pair approximating 50 mm on the one plate (Fig. 4) and 80 mm on the other plate. These distances were measured in three different photogrammetric instruments: a Wild Autograph A8 in Lund, and a Wild StK-1 at the Department of Photogrammetry, Royal Institute of Technology, Stockholm, Sweden, as well as in a Zeiss Stecometer (VEB Carl Zeiss, Jena, East Germany) at the Institute for Surveying and Photogrammetry, Technical University of Denmark,

Copenhagen, Denmark. The latter two, both stereocomparators, have an accuracy near $1\text{ }\mu\text{m}$.

When comparing measurements, the root mean square difference between distances measured in Stockholm and Copenhagen was $7.6\text{ }\mu\text{m}$. An identical difference, $7.6\text{ }\mu\text{m}$, was obtained when comparing two different measurements, both performed in Copenhagen. A single setting was made of each ball. The distance differences obviously reflect setting inexactness and not instrument differences. Also, it was found that the same repeatability was obtained using the Wild Autograph A8 in Lund. Here, a root mean square difference of $6.1\text{ }\mu\text{m}$ was registered in one test, each distance determination being the mean of six measurements.

The above-mentioned calibration measurement values were taken as test plate standards, which were subsequently compared to distances measured from stereoradiographs employing different film/screen combinations using either a projective transformation (PT) or a similarity transformation (ST) for image reconstruction (Table 1). The absolute values in this table refer to the root mean square of such comparisons calculated from Formula 5 below, with the denominator $n=9$. The relative values, on the other hand, refer to a comparison between two different tests, with distances determined only from radiographs, one with the markers at height 5 mm above the lower plate of the calibration cage and the other at height 70 mm. Here, Formula 5 is applied with denominator $2 \times n$ ($n=9$). The factor 2 was introduced with the intention to give a precision value for one distance, based on comparisons of two distances, both with approximately the same error. By radiographing in two completely different positions, distance differences give a good representation of possible variations in radiographic examinations of biological objects. Table 1 shows that the recently used mammography film gives lower precision than the previously employed film/screen combinations. Nevertheless, the error in distance measurements was about $10\text{ }\mu\text{m}$.

Suboptimal object marker positioning

An important application for the present method has been the registration of longitudinal bone growth. Marker placement, both in bone tissue and at radiography, should fulfill certain criteria. Placed on both sides of a physis intending to register longitudinal growth, the line connecting the markers is to parallel the growth direction. The angle between such a marker line and a line in the actual growth direction is denoted by α in Fig. 5. The approximate relation between growth (dl) and distance change between markers (ds) is

$$dl = \frac{ds}{\cos \alpha}, \quad (4)$$

which follows from the definition of cosine applied to the "small" triangle with sides dl and ds (Fig. 5). As $\cos \alpha = 1$ for $\alpha = 0^\circ$, marker distance changes then represent growth, but for $\alpha \neq 0$ ($\cos \alpha < 1$) it is evident that the measured distance change is lower than the actual growth. Thus, for example, for $\alpha = 10^\circ$ ($\cos 10^\circ = 0.985$) or $\alpha = 20^\circ$ ($\cos 20^\circ = 0.940$) growth is underestimated by 1.5 % and 6 %, respectively.

Regarding the placement of the marker line in the calibration cage, this is favorably placed parallelly to the FM plane. The formula (1) describing errors in object coordinates due to errors in CP coordinates shows the errors to be the same for a certain height coordinate; thus, for a marker line parallelling the FM plane, coordinate differences (and subsequently distances) are not influenced by such errors. After correct calibration, where also d_x and d_y are adjusted and residual errors are small; the distance errors for oblique lines due to calibration inexactness are insignificant.

Applications to experimental bone growth

The present method of RSA has been applied to many experimental, as well as clinical fields in e.g. orthopedics, orthodontics, pediatrics and neurosurgery. The aim has been to describe and analyze growth, form changes, joint mobility and so forth. Methodological accuracy is of the utmost

importance, in particular when studying slow or slight quantitative growth or joint movements. Also, the quality of the procedure must not be influenced by positional changes of the object between measurements.

To illustrate the validity of RSA measurements, a compilation of experimental data from various investigations of rabbit cranial vault and tibial growth have been listed in Table 2. These tests were carried out in such a way that two separate stereo examinations were performed in two different positions of the investigated object. After the film analysis, the results of the two exposures were compared according to the formula:

$$ME = \sqrt{\frac{(d_1 - d_2)^2}{(2 \times n)}}, \quad (5)$$

where ME = mean error

$d_1 - d_2$ = difference between measurements

n = number of measurements.

The denominator n was used for calculations of growth and kinematic variables as these, in applications, are calculated from two examinations. On the other hand, for length and volume calculations, the denominator 2 x n was used since these only need one examination.

The computer programs used in the presented applications are termed GROWTH, VOLUME and KINEMA. GROWTH is a small program, which calculates 3-D distances between implanted markers from their RSA-determined 3-D co-ordinates, using the 3-D Pythagorean theorem. Furthermore, distance changes and relevant statistics are presented. In the fairly comprehensive program VOLUME a specific polyhedron is defined with corners at the implants. In an actual case these were situated in the frontal, parietal and temporal bones (Fig. 6 A). The polyhedron is constructed as the closest to a convex polyhedron in a specific sense (Claesson et coll. 1978) from the given marker configuration.

As to the kinematic analysis (performed in a comprehensive program, KINEMA), this requires a more extensive review. Three stable bone markers, not on a line, constitute a rigid-body model (RBM) and thereby represent a segment (bone) in calculations of motion (Fig. 6 B). One segment is considered fixed. At each evaluation this RBM is reoriented to its original position in the laboratory coordinate system. The segmental spatial displacements are described as rotations about and translations along the three cardinal axes, following classical kinematic principles for rigid-body movements. Translations are given for the center of gravity of the RBM or for separate implants, both as their components in the directions of the cardinal axes and as total translation, i.e. the distance calculated from these components using the 3-D Pythagorean theorem. Consequently, a complete mapping of growth patterns for a specific or for several bones relative to a selected reference bone can be undertaken with high accuracy.

DISCUSSION

Roentgen rays travel in straight lines as already pointed out in the original publication by Röntgen (1895). Also, he observed no evidence of refraction at the surfaces between different media. Later investigations, though, have shown a deviation from the refractive index of 1 by a magnitude of $1 \cdot 10^{-5}$ when rays of the wavelengths most commonly used in diagnostic radiology are refracted by, for instance, glass. Thus, the ray displacement is in the order of magnitude of 1 μm or less when traversing calibration objects as those presently applied (Selvik 1974). Consequently, the geometry by which an object is transformed to a film image at a roentgen exposure from a single focus is that of a central projection. Hereby, the bundle of rays can be reconstructed from given positions of certain test markers.

The roentgen foci to film arrangement, where the line joining the two foci parallels the film plane, is a realization of the photogrammetric "normal case" (Hallert 1970). The focus to focus distance was about

40-60 cm and the focus to film distance about 100 cm, corresponding to an angle between the central rays of $25^{\circ} - 35^{\circ}$. Alternatively, a configuration with symmetric, convergent bundles of rays, their central rays at right angles to each other (biplanar technique) can be used (Selvik 1974, Lippert et coll. 1982). The present geometry has a lower resolution in the vertical direction than the biplanar case (films at right angles), where "height" is determined with the same accuracy as other measures. Thus, for photogrammetry of objects with extension in all three dimensions, the biplanar technique is to be preferred. For measurements of distances, though, which can mainly be restricted to one plane, the present calibration equipment is advantageous. This cage can be constructed of glass being thermostable (coefficient of thermal expansion approximately $8 \cdot 10^{-6} / ^{\circ}\text{C}$) compared to plexiglass ($75 \cdot 10^{-6} / ^{\circ}\text{C}$), but also resistant to changes of relative humidity. This entails a distance change of only $1.6 \mu\text{m} / ^{\circ}\text{C}$ for the 200 mm long FM distance of the calibration cages lower plate.

The quality of the primary reconstruction and of some tests and applications were presented to demonstrate what can be achieved by using standard roentgen equipment and a generally available photogrammetric instrument (Wild A8) handled by careful but not specifically trained laboratory personnel. Measurements of distances could be performed with an accuracy close to $10 \mu\text{m}$. The lower precision of the recently used mammography film can only be explained by a decreased accuracy of the measuring instrument during the lapse of eight years since the initial tests. The time gained by using a similarity transformation instead of a central projection is not coupled to a substantially diminished accuracy in determining distances as illustrated by both technical tests and applications.

The calibration of the calibration cage itself was performed in three steps. The first one, i.e. the measurement of marker coordinates in the two planes, is straightforward and here the thermostability implies that the accuracy of measured coordinates is preserved in the applications. The second step, the determination of rotations between the plates, can be

highly accurately accomplished but it was found that stereo model coordinates are not appreciably influenced by the rotation correction when amounting to values below one degree of angle (Selvik 1974). On the other hand, the final third step, the determination and correction of translation between the plates, is required to obtain a true 3-D reconstruction of object coordinates.

In clinical applications to fibular and tibial growth, it was found that the angle between a line joining the markers and a perpendicular line to the growth plate never exceeded 10° . When implanting markers in the two tibial epiphyses of a rabbit leg, the marker line well represents the bones longitudinal axis. Thus, registered distance changes do represent growth well. However, when studying longitudinal growth at the cranial vault sutures, the marker placement can not always be on a line perpendicular to the sutural line due to the sutures growth pattern. Consequently, distance changes do not exactly reflect sutural growth. But, as discussed in connection with Formula 4, the underestimation will be slight, 6 % for 20° deviation, which was the maximally observed in this case.

At the calibration of the 3-D test object this object was calibrated simultaneously with the calibration equipment itself. Thus, nothing is needed from absolutely determined space coordinates in advance. By the "trick" of rotating the test object 180° , systematic errors in preliminary obtained object coordinates can be eliminated, and a correction to preliminary cage coordinates is determined. Thus, in one operation, calibration equipment intended for further investigations and a test object for evaluations of radiographic measurements have been successfully produced. It can also be noted that for determining space coordinates of radiopaque markers with high accuracy there is, to our knowledge, no other procedure available.

The technical procedures involved in the construction of a roentgen stereophotogrammetrical system which can be actualized in all radiographic

institutions have been outlined in detail. With the advent of efficient microcomputers and the forthcoming of small size coordinate tables coupled to these computers, this technique should be an attractive prospective to radiologists for applications in, for instance, highly accurate measurements of skeletal growth.

SUMMARY

The principles for a system of roentgen stereophotogrammetric analysis (RSA) for experimental and clinical applications are presented. Only the positions of implanted reference markers in the studied object are measured. Tantalum balls, 0.5 or 0.8 mm in diameter, are used as reference markers. Standard roentgen equipment, permitting simultaneous exposure from two roentgen tubes, is employed. The calibration equipment, a glass calibration cage containing radiopaque markers, introduces a rectangular laboratory coordinate system in the roentgen investigation. The calibration of the calibration equipment itself and the obtained methodological accuracy in determining distances, volumes and movement parameters is described for various film/screen and marker combinations.

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Table 1. Absolute and relative accuracy of distance determinations from a 2-D test object (Fig. 4) obtained using projective transformation (PT) or similarity transformation (ST) for different film/screen combinations.

Film/screen combinations	Transformation	Absolute (μm)	d.f.	Relative (μm)	d.f.
AG D7/ -	PT	11	36	6.4	18
Standard/ /Rubin	PT	13	36	5.4	18
Mammography/ /gadolinium- oxysulphide	PT	12	36	13	18
	ST	16	36	14	18

Table 2.

Presentation of the RSA accuracy for various analyses and experimental investigations.

Axes: X = transversal, Y = longitudinal, Z = vertical. m/g = mammography / gadoliniumoxysulphide.

PT = projective transformation, ST = similarity transformation.

	Rabbit cranial vault growth		Rabbit tibial growth	
	Growth (Alberius & Selvik 1982)	Kinema (Alberius & Selvik 1983)	Volume	Growth (Aronson et coll. 1978)
Marker diameter (mm)	0.8	0.8	0.8	0.5
Number of animals	7	4	8	8
Number of markers	89	88 (18 segments)	111	32
Number of distances	529	-	-	-
Number of segment combinations	-	11	-	-
Methodological error	growth 21.0 μ m length 14.8 μ m	rotation axis: translation axis: X 50 μ m Y 26 μ m Z 84 μ m	0.48 %	growth 13 μ m
Film/screen combinations	m/g	m/g	m/g	D7/-
Transformation	ST	ST	ST	PT

7 •	8 •	9 •
-65.773, 199.508	0.0, 199.328	64.211, 198.993
-65.772, 199.493	0.0, 199.325	64.225, 198.997

4 •	5 •	6 •
-65.055, 99.544	-0.593, 99.829	64.263, 99.498
-65.064, 99.558	-0.588, 99.822	64.258, 99.497

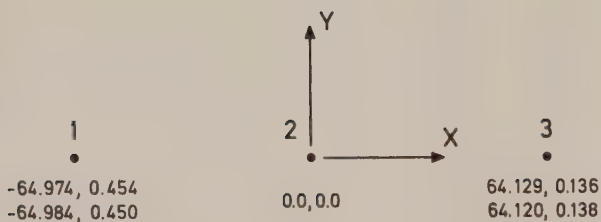


Fig. 1 Coordinates of the lower plate (fiducial marks) from two marker calibrations.

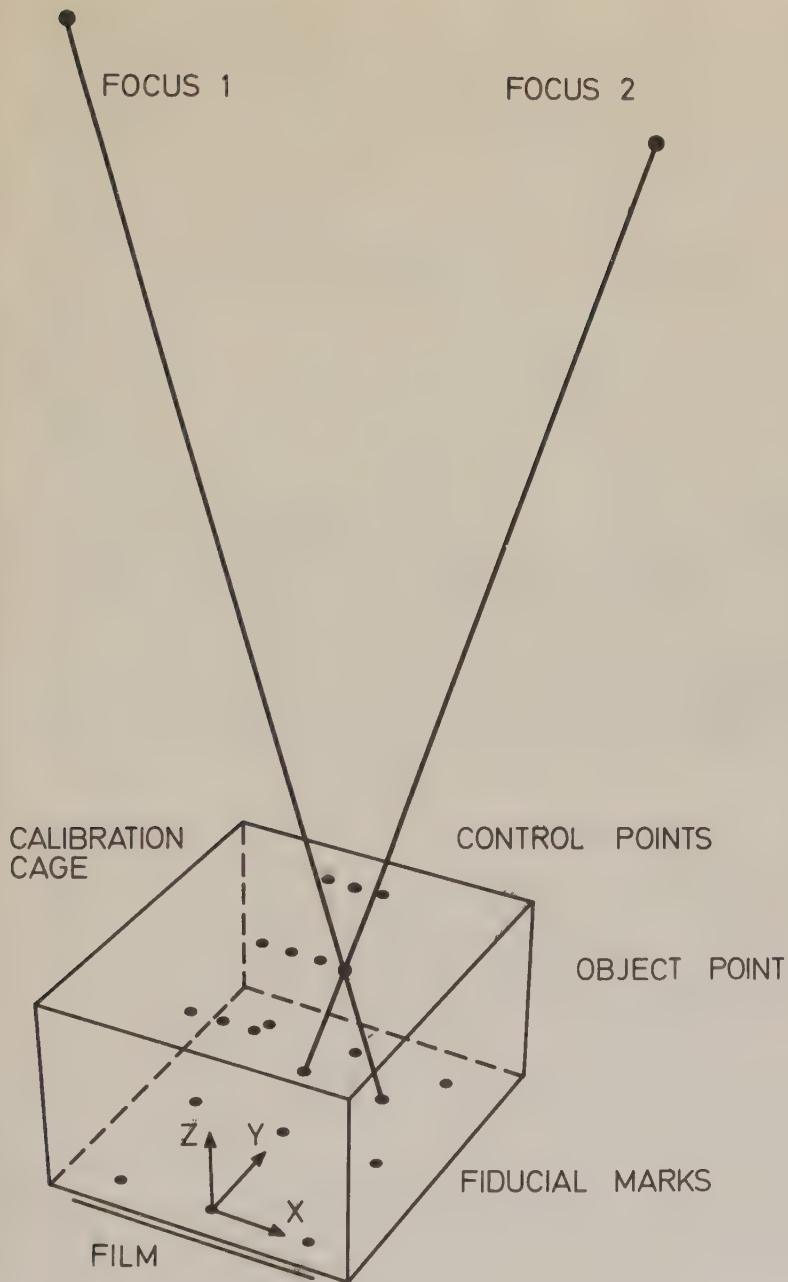


Fig. 2
The roentgen
stereophoto-
grammetric equ-
ipment. The fo-
ci are placed
about 0.4-0.6
m apart and 1.0
m above the
film. Lower
plate markers
are positioned
as shown in Fig.
1, the upper
plate markers
form a rectang-
le, 40 x 170 mm.

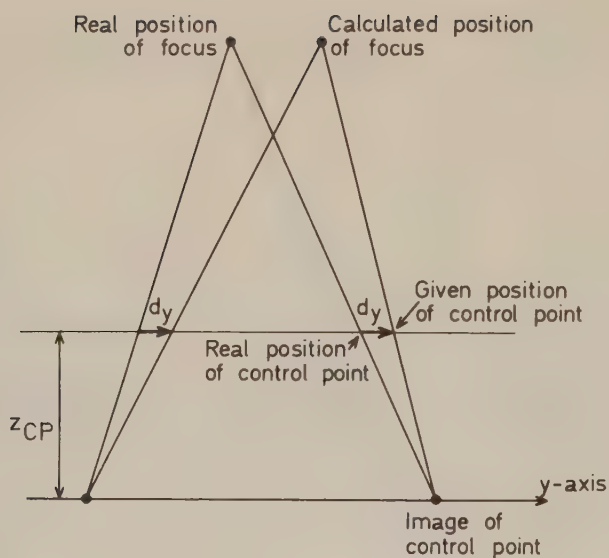


Fig. 3 Demonstration of the influence of an error d_y of given CP coordinates on calculated position of the roentgen focus. The distance between the FM:s' and CP:s' planes is z_{CP} .

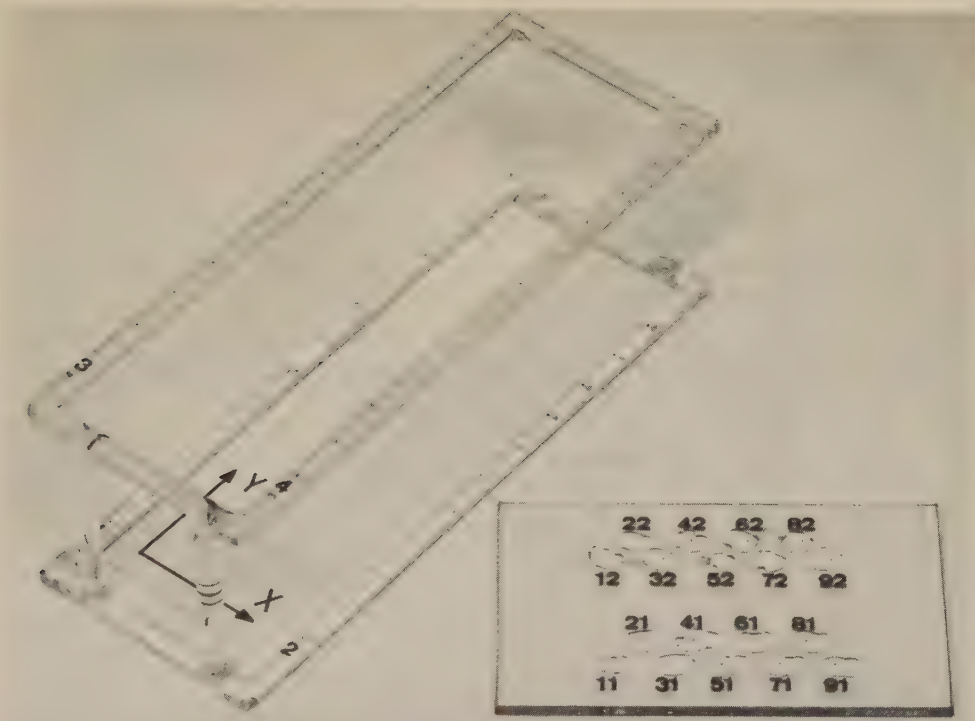


Fig. 4 The 3-D test object (left) and the plate for determining length accuracy (right). Each contains tantalum balls, 0.5 mm in diameter, glued to the glass with epoxy glue. The test objects contain 44 and 18 markers, respectively. Of the 44 markers 11 lie on each of the four long bars. The directions of coordinate axes attached to the 3-D test object are shown, the z-axis (not indicated) points upwards and is perpendicular to the x- and y-axes. The distances, about 50 mm long, between markers 11-12, ..., 91-92, are those investigated for the test plate.

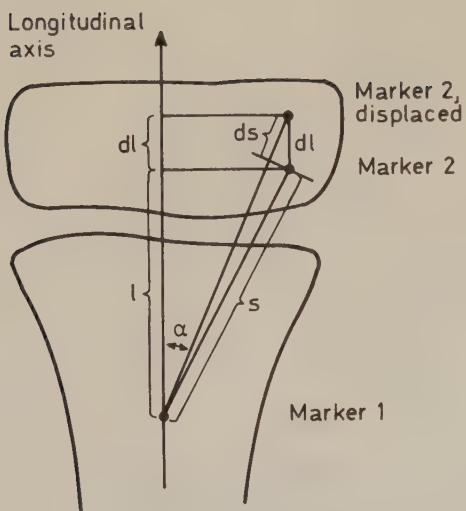


Fig. 5 Illustration of the effect of suboptimal object marker positioning. The constructions demonstrate the increase of distance (ds) between markers not separating along the actual axis of longitudinal bone growth. Longitudinal growth marked dl .

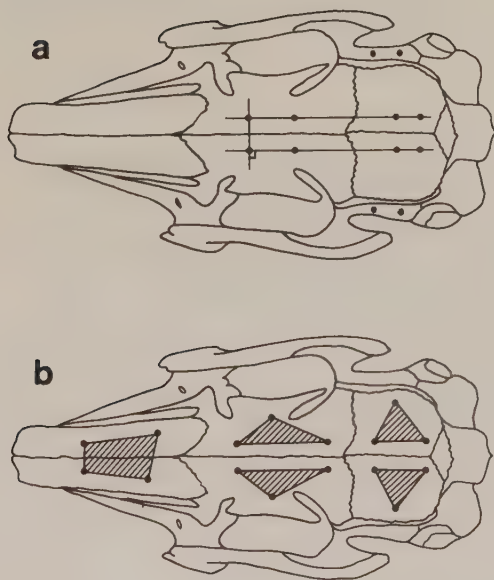


Fig. 6 Illustrations of the rabbit cranial vault implantations. a/ A polyhedron for volumetric calculations is obtained by uniting the markers. b/ The construction of rigid-body models (RBM; stippled areas).

Bone Reactions to Tantalum Markers A Scanning Electron Microscopic Study¹

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Key Words. Tantalum · Metallic implant · Bone reactions · Scanning electron microscopy · Roentgen stereophotogrammetry

Abstract. To elucidate intramembranous bone reactions to tantalum bone markers, 0.8-mm tantalum balls were implanted in the craniofacial region of 9 male New Zealand white rabbits. After 1, 2, 4 and 16 weeks the animals were sacrificed for SEM. Implant stability for these intervals was controlled by roentgen stereophotogrammetry. Fibrocytes and deposited fibers were seen to immediately adhere to the ball surface, getting retention in its porosities. A successively increasing bony support was observed to have intimate junctions without intervening fibers or soft tissues. No apparent differences in bone reactions were seen between the neurocranial and splanchnocranial bones. Osseointegration confirmed the inertness of tantalum to bone tissue and strongly confirms its suitability for implantation in bone.

Introduction

The extensive search for ideal alloplastic replacements for skeletal defects has necessitated studies on interactions between implant materials and their biologic environment. Several materials have been investigated.

¹ Scanning electron microscopic examinations performed at the Department of Structural Zoology, University of Lund.

Burke [1940] introduced tantalum into surgery. Since then, its excellent mechanical properties, bioinertness and resistance to corrosion (destruction caused by the tissue environments) have allowed its application in numerous forms and usages.

Recently, the possibility of obtaining osseointegration – a direct contact between living bone and implant – using tantalum, was reported. Grundschober et al. [1980] examined four tantalum and one titanium

endosseously implanted dental screws which had been implanted in man for 8–12 years, and found a direct bone-to-implant contact without encapsulating soft tissues.

Nevertheless, a few complications have been observed with tantalum, though some of these have been questioned. *Delarue et al.* [1944] implanted tantalum foil in the dog subdural space over a traumatized cortical area. Thickening of the dura mater and, in many, a similar but slighter reactive thickening of the underlying arachnoid membrane was present. This experiment was repeated by *Vieth et al.* [1966], who registered no similar tissue reactions. *Bailey et al.* [1952] experimentally introduced tantalum powder into the basal cisterns and obtained severe reactions with fatalities. *Smith* [1973], on the other hand, injected tantalum powder into the cervical subarachnoid space without any ill effects. He considered *Bailey et al.*'s [1952] results to be due to the quantity of tantalum injected (about 0.5 g).

Meirowsky et al. [1950] observed development of epidural granulomata when performing tantalum cranioplasty in the presence of infection. Furthermore, *Werman and Rietschel* [1981] reported a case of chronic urticaria from tantalum staples, and proposed a type I reaction or a direct histamine release via nonimmunologic mechanisms.

Different types of bone markers have been applied to define anatomical landmarks when distinct skeletal marks are unavailable [review: *Sarnat and Selman*, 1978]. *Björk* [1963] introduced the use of tantalum implants into the human craniofacial skeleton to improve reorientation in roentgen cephalometry, after originally using vitallium [*Björk*, 1955].

Tantalum was also considered suitable

for roentgen stereophotogrammetric analysis of growth (RSA), [*Selvik*, 1974], using a specially developed implantation instrument [*Aronson et al.*, 1974]. An initial marker instability, though, has been noted for different types of bones, both clinically [*Rune et al.*, 1979, 1981; *Bylander et al.*, 1981] and experimentally [*Aronson and Hansson*, 1976].

The present study compares the neurocranial and splanchnocranial bone implant reactions and aims to elucidate the histologic basis for RSA-verified initial instability.

Materials and Methods

9 male New Zealand white rabbits were used. The animals were maintained on a 12-hour light-dark cycle at 20°C with a relative humidity of approximately 40%. Standard pellets and water were provided ad libitum. At age 4 weeks the rabbits were anesthetized by intramuscular neuroleptanalgesia (Hypnorm® vet.: Fluanizon and Phentanyl, 10 and 0.2 mg/ml, respectively; 0.6 ml/kg body weight). The scalp was shaved and the surgical area cleaned with alcoholic chlorhexidin (5 mg/ml).

The experimental site was exposed by a para-midline skin incision from the parietal to the nasal region. The scalp and underlying tissues were reflected laterally and the nasal, frontal and parietal bones identified.

At least two 0.8-mm sterile tantalum balls were implanted in each bone, using a specially designed instrument [*Aronson et al.*, 1974], to make possible roentgen stereometric control of ball stability. Care was taken to minimize trauma to the periosteum so as to diminish influence on bone regeneration. The incision was closed with 4/0 Ethicon sutures and the surgical site finally covered with Nobecutan® spray. The animals were sacrificed according to the schedule shown in table I.

A diamond-edged wheel attached to a dental drill was used to remove the craniofacial bones (nasal, frontal, parietal) from each specimen, which were subsequently examined under a dissecting microscope. Tantalum balls properly implanted, i.e. embedded in bone,

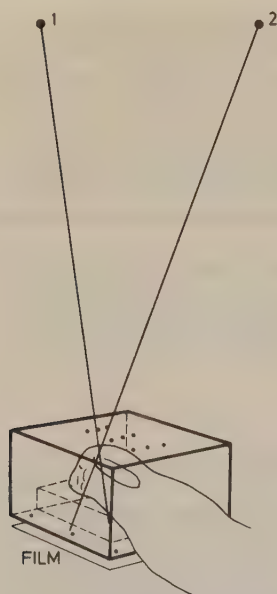


Fig. 1. Roentgen stereophotogrammetry. The rabbit head is placed on a support in the calibration cage and is simultaneously radiographed by two roentgen tubes (focus 1 and 2). Cage reference markers in the upper and lower plane indicated.

were prepared for scanning electron microscopy (SEM).

SEM Procedure

Half of the bone specimens were immersed in 2.5% glutaraldehyde in 0.2 M cacodylate buffer, pH = 7.2, the remainder were air-dried. Specimens were fixed for 3 h (room temperature; RT), washed in cacodylate buffer (RT), dehydrated in graded series of alcohols and critical point dried using CO₂ as a transitional fluid.

The markers were localized roentgenographically, after which they were exposed for microscopic study by fracturing the bone. However, not all markers could be found by fracturing. The specimens were then mounted on SEM stubs, coated with gold/palladium in a vacuum sputter and examined in a Cambridge stereoscan Mark II A scanning electron microscope.

Roentgen Stereophotogrammetric Analysis (RSA)

The principles of roentgen stereophotogrammetry and methodological errors have been presented earlier [Selvik, 1974; Alberius and Selvik, 1982].

The rabbit was examined in a glass calibration cage containing metal reference markers (fig. 1). Two roentgen tubes, positioned with an approximate focus to focus distance of 1 m, simultaneously exposed a vacuum-packed envelope enclosing a mammography film and a gadoliniumoxysulfide intensifying screen. The films were analyzed in a precision instrument for aerial photogrammetry (Wild Autograph A 8), recording the 2-D coordinates for the calibration cage reference markers and the tantalum ball centers. The 3-D coordinates of the tantalum bone markers were obtained by computer processing.

By implanting at least two balls in each bone, comparison of distance changes, and thus stability (= change $\leq 42 \mu\text{m}$), was enabled.

Table I. Sample distribution

Group	Duration, weeks	Markers		Nasal	Frontal	Parietal	Animals
		implanted	SEM				
I	1	29	13	7	3	3	2
II	2	27	17	6	5	6	2
III	4	14	8	2	4	2	1
IV	16	<u>103</u>	<u>29</u>	8	7	14	<u>4</u>
		173	67				9

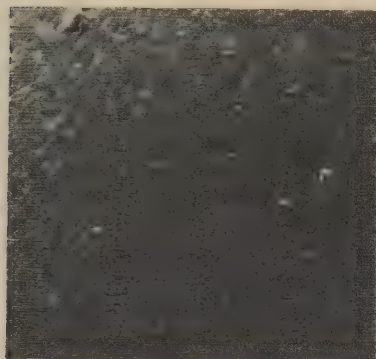


Fig. 2. Tantalum ball surface. $\times 240$.

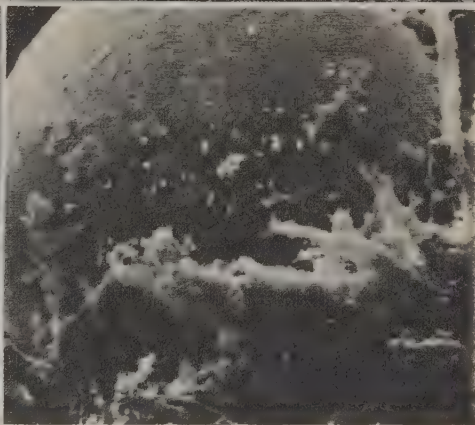
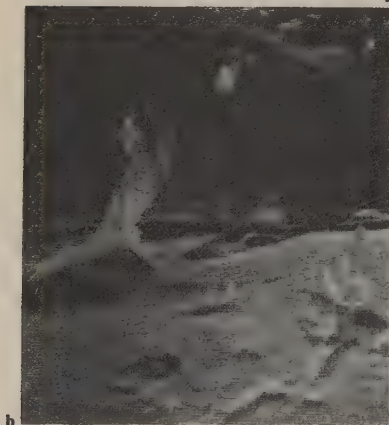


Fig. 3. 1 week examination. **a** Ruptured membrane of fibroblasts (FB) covering tantalum marker surface. Air-dried parietal bone specimen. $\times 120$. **b** Cellular

projections adhering to marker porosities (area outlined in fig. 3a). $\times 600$. **c** Intercellular fibers showing finger-like projections. Nasal bone. $\times 120$.

Results

The surface of an unused tantalum ball is illustrated in figure 2.

Week 1 (fig. 3)

An incision parallel to the implantation incision permitted folding the scalp over. The soft tissues appeared macroscopically

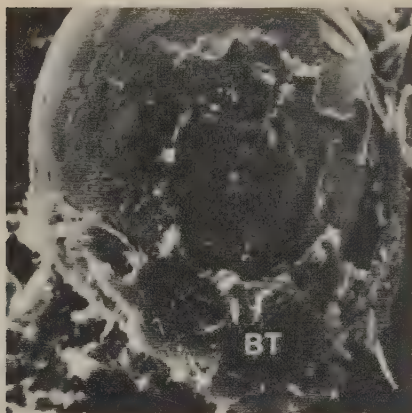


Fig. 4. 2 week examination. Bone tissue (BT) now adhering more firmly to marker. Parietal bone. $\times 90$.

healed. The implantation holes in the external cortical plate persisted, clearly visible, but were covered by a denser calvarial periosteum. Several balls were lost upon fracturing, and seemed only loosely attached to the bone tissue.

SEM. A zone consisting of fibroblasts surrounded the implant. These often adhered to the rough tantalum surface, as did intercellular fibers. The fibers showed no obvious orientation, having finger-like projections, of which some seemed to adhere to porosities in the balls. However, the fibrous membrane was often ruptured, exposing the ball surface. Random bone processes were seen to touch the implant.

Week 2 (fig. 4)

The tantalum balls were covered by a thin film of bone, but still the external laminar bone holes were easily detected. The bone appeared to retain the balls more firmly, as only a few balls were lost upon

fracturing the bone. The calvarial periosteum remained dense.

SEM. The SEM picture closely resembled that of week 1. Additionally, many irregularly dispersed bone spicules were observed, contacting the implant.

Week 4 (fig. 5)

The holes in the external cortical plate were still discernible. The balls appeared to be covered by compact bone. The periosteum still appeared dense.

SEM. A dense irregular network of fibers clinged to the implant. Numerous scattered bone bridges were seen in direct contact with the tantalum surface.

Week 16 (fig. 6)

The ectocranial bone table covered all the tantalum balls, making the implantation sites impossible to distinguish. Some implants could scarcely be discerned, especially through the spongy external lamina of the parietal bone.

SEM. The implants seemed firmly attached to the bone spicules, which structurally appeared similar to cortical bone. Frequent direct bone to implant contacts without detectable intervening substance were observed. These junctions were interspersed with solitary localized intervening spaces. Soft tissue was sparse. In most cases, fracturing did not seem to expose the ball.

Disregarding basic histological differences, no dissimilar reactions toward the tantalum implants were observed during the investigation. Air-dried bone specimens exhibited slight shrinkage as compared to glutaraldehyde fixed, thereby magnifying the intervening ball to bone spaces. Surface comparisons of new and implanted balls revealed no signs of corrosion.



Fig. 5. 4 week examination. Area of closely located bone bridges in direct contacts with tantalum surface. Frontal bone. $\times 200$.

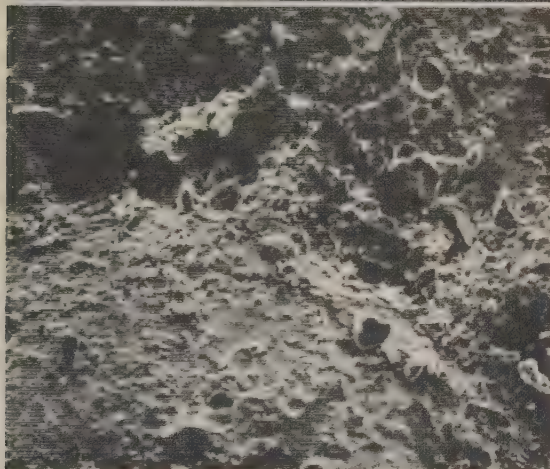


Fig. 6. 16 week examination. Bone tissue firmly attached to tantalum marker, the bone-to-implant contact thus withstanding the bone fracturing procedure. Parietal bone. $\times 240$.

Stereometric Analysis

To test whether RSA-verified instability could be characterized histologically, stereometric and histological examinations were performed separately, and then compared.

Stereometric instability was registered primarily in the nasal bones. In the majority of cases, almost complete bone penetration into the nasal or the cranial cavity was observed. This was probably caused by the spring in the implantation instrument, which apparently was too powerful for insertion

into this thin bone. However, no histological differences were observed between completely embedded stable and unstable balls.

Only one implant, from the parietal bone, totally penetrated the calvarial bone during the observation period.

Discussion

This study deals with bone reactions to tantalum implants. The specimen preparation implied fracturing the bones to uncover

the implants. This procedure may have affected the interface between the markers and the surrounding tissues. Nevertheless, fibrocytic invasion resulting in a fibrous network was seen to occur rapidly, the finger-like processes adhering to the implant's surface porosities. This adhesion was disrupted, unable to withstand the forces inherent to the fracture procedure. The bone healing process seemed to progress undisturbed towards osseointegration, showing a similar response in bones in the facial skeleton and the neurocranium.

This acceptance of tantalum implants by bone tissue agrees with previous reports. *Burke* [1940] found no detectable macroscopic, microscopic or roentgenologic evidence of bone or soft tissue reactions to tantalum screws and plates inserted into dogs and rabbits tibiae and femurs. In addition, *Carney* [1942], studying dog tibiae, observed roentgenologically an increased bone density.

Aronson and Johnsson [1976] registered no or only slight fibrosis in rabbit long bones and human fibulae, while *Grundschober et al.* [1980] described osseointegration of dental tantalum implants.

According to *Albrektsson et al.* [1981], osseointegration is dependent on the implant material, its design and finish, healthy bone tissue, minimal surgical trauma and stability.

Some parameters need further comment. In this study, the rather rough ball surface provided possibilities for initial rapid cellular adhesion [*Albrektsson et al.*, 1981], which is a prerequisite for the development of stability. As for the surface structure, also the surface energy has been reported to influence the tissue response [*Meenaghan et al.*, 1979].

Bone structure at the implantation site, whether cortical or medullary, seemed to be of minor importance for proper osseointegration, as 'corticalization' of spongy bone surrounding a metallic implant tends to occur [*Breine and Brånemark*, 1980]. The thinness of rabbit calvarial bones at the time of implantation, inevitably causes both diploic and compact bone to be in intimate contact with the implant, thereby resulting in a change of bone structure.

The initial marker instability observed by roentgen stereophotogrammetry could not be characterized histologically. Instead, this may be due to the healing process, starting with irregular fibrous adhesions and only sparse bony support, but may also result from intrinsic bone elasticity. Nevertheless, tantalums full acceptance by bone tissue supports its use as a reliable biometrical implant. Also, initially loose markers have been observed to stabilize [*Alberius and Selvik*, 1982].

Interestingly, despite the normally extensive remodelling of the cranial vault bones [e.g. *Cleall et al.*, 1968, 1971; *Hong et al.*, 1968; *Hoyte*, 1966, 1971], with its inherent risk of ball penetration to the dura mater and subsequent loss of bony support, the extent of bone-implant adhesion seemed to remain unaffected by these processes.

After coronal suture immobilization, *Foley and Kokich* [1980] consistently observed the occurrence of ectocranial interbone fusion in rabbits less than 8 weeks of age. They concluded that such bridging does not occur at older ages due to a diminished ectocranial bone deposition once active neurocranial expansion has ceased. To the contrary, it was observed here that a continuation of external deposition beyond age 8 weeks took place, since the holes left

by marker implantation did not completely disappear until 20 weeks of age. Furthermore, after age 8 weeks, neural tissues increase by 20% to obtain adult size [Harel et al., 1972].

Despite the inertness of tantalum reported by several investigators [e.g. *McFadden*, 1969; *Meenaghan* et al., 1979; *Burke*, 1940; *Smith*, 1973; *Spurling*, 1943; *Samuels* et al., 1966; *Nadel* et al., 1968; *Grundschober* et al., 1980] and further corroborated in this study, adverse reactions may occur. Intradermal testing has verified a case of chronic urticaria to be caused by tantalum surgical staples [Werman and Ritschel, 1981]. Thus, though extremely infrequent, side effects must be kept in mind.

To conclude, tantalum markers successfully integrate to a stable position in bone. The RSA-verified initial instability may be explained by the irregular, mainly fibrous support of the implant. Corrosion could not be verified by SEM. Nevertheless, awareness of eventual side-effects is needed.

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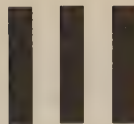
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ROENTGEN STEREOPHOTOGRAMMETRIC ANALYSIS OF GROWTH AT CRANIAL VAULT
SUTURES IN THE RABBIT

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Key words: Growth, bone, craniofacial, suture, metallic implant,
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Running head: Rabbit cranial vault growth

ABSTRACT

Biometric characterization of rabbit cranial vault bone separation was attempted to further elucidate sutural growth mechanisms. The internasal and frontonasal sutures, connected to the facial skeleton, were also investigated. 17 male New Zealand white rabbits were studied from age 4 to 21 weeks using roentgen stereophotogrammetry with tantalum implants. A uniform cranial growth pattern with successively decreasing rates was observed, as were different growth rates at the neurocranial bones relative to those of the facial skeleton. Antero-posterior length increase was due mainly to bone separation at the frontonasal suture. Growth at the sagittal suture was minimal. Minor rate fluctuations and compensatory rebounds after periods of reduced growth were continuously observable. Small left-right differences within each individual were registered. Oscillations in growth rate across adjacent sutural areas and occasional negative growth values imply that bone separation at sutures demonstrates an alternating pattern of translations and rotations. The experimental data demonstrated the plasticity and variability of normal cranial vault growth.

INTRODUCTION

The processes of vertebrate cranial growth have been extensively studied, but still the function and nature of the cranial suture is disputed.

Cranial sutures are arranged in relation to one another so as to permit compensatory increases in cranial length, width and height. Being a remnant of the original membranous cerebral capsule, the cranial vault suture remains an adaptable modulating shock-absorbing syndesmosis between adjacent bones to prevent any undue separation and allows for spatial adjustments. The sutural adaptability is also demonstrated by minor variations in its location after experimental trauma (Moss, 1954; Girgis and Pritchard, 1958; Sarhat, 1958; Sirola, 1960; Ryöppy, 1965).

Exact measuring techniques are required to determine short period skull growth. Various methods have been employed, e.g. serial roentgenographic measurements, vital staining and autoradiography. Yet, many questions relating to the basic nature of the neurocranial suture remain to be solved

and detailed quantitative information on normal calvarial sutural behavior is incomplete. By roentgen stereophotogrammetric analysis using metallic implants (RSA; Selvik, 1974)) new information may be obtained with high accuracy. Thus, the mechanisms and characteristics of sutural growth can be investigated.

MATERIALS AND METHODS

Animals

17 male New Zealand white rabbits, aged approximately four weeks (mean, 30.5 ± 2.5 days; mean weight, 0.613 ± 0.136 kg) were used. The animals were kept at 20°C (40% relative humidity) with light from 6 a.m. to 6 p.m. Standard pellets and water provided *ad libitum*. To avoid seasonal variations experiments were performed during the whole year. The rabbits were followed until age 21 weeks (145.2 ± 7.4 days) since, according to Engdahl (1972), a levelling off in their growth curve is shown at an age of five months.

Operative technique

The animals were anaesthetized by intramuscular neuroleptanalgesia, Fluanizon and Phentanyl, 10 and 0.2 mg/ml, respectively; 0.6 ml/kg body weight (Hypnorm[®] vet.). The rabbit head was shaved, after which an antiseptic solution (alcoholic Chlorhexidin, 5 mg/ml) was applied to the surgical site. A para-midline scalp incision from the parietal to the nasal region was made. The skin and underlying tissues were reflected laterally and the calvarial sutures identified. Tantalum bone markers (0.8 mm in diameter) were implanted in the nasal, frontal and parietal bones, at least two in each bone (=segment), using a modified version (new release mechanism) of a specially constructed instrument (Aronson et al., 1974). To minimize the risk of uncontrolled penetration into the cranial bone, cylindrical stop-blocks were mounted on the needle, leaving respectively 0.5 and 0.75 mm of the tip exposed (Fig. 1). The markers were implanted along bilateral rostral-caudally oriented lines parallel to the median plane; the connecting line between each bilateral pair being at a right angle to the midline (Fig. 2). 7 rabbits received an extra pair of bone markers across the frontonasal and coronal sutures bilaterally (Fig. 2). In general, care

was taken to minimize periosteal manipulation. The incision was closed with interrupted 4/0 Ethicon sutures and covered with Nobecutan[®] spray. The animals were left to recover on a warm electric blanket.

Roentgen stereophotogrammetric examinations

The animals were examined in a glass calibration cage supplied with tantalum reference markers (Fig. 3). Two simultaneously exposing roentgen tubes were positioned with an approximate focus to focus distance of 0.4 m and a focus to film distance of 1.0 m. Exposure data for a rabbit cranial vault examination were 48-51 kV and 4-16 mAs, depending on age.

Stereo roentgenograms were obtained on the day of implantation (age 4 weeks) and at ages 6,7,8,9,10,12,14,16 and 21 weeks. Weight control was carried out at each examination.

Analysis of data

The films were digitized in a precision instrument for aerial photogrammetry (Wild Autograph A8). The 3-D coordinates of the bone markers were obtained by computer processing. The following criteria were used to standardize the evaluation:

1. Only markers showing intrasegmental stability throughout the investigation were accepted.
2. The two most ideally placed markers on each side of a suture, according to the principles of implantation, were selected.

To reduce the influence of methodological errors (see below) on growth rate values, the coronal suture was observed for minimally two-week intervals, thus omitting weeks 7 and 9. The sagittal suture, whose growth between succeeding measurements approached the methodological error, was analysed only for weeks 4,7,13 and 21.

Due to insufficiently accurate implantation procedures and occasional marker loss, a few analyses were impossible to undertake, explaining the different numbers of animals in various sutural analyses.

To avoid influence from unrepresentative growth values, e.g. due to abortive roentgen examination or major illness (as determined by weight

loss), some rate values were excluded. These exclusions do not affect the statistical evaluation due to its nature.

For reasons of simplicity the sutures studied are referred to as calvarial or cranial vault sutures (neurocranium), despite both the inter-nasal and frontonasal sutures being connected to the rabbits facial skeleton (viscerocranium).

Accuracy of the method

To test the accuracy, two exposures with different orientations of the skull were obtained in 7 animals.

Thereafter, measurements of these two radiographs were compared with respect to the differences of the implant distances according to

$$ME = \sqrt{\frac{\sum d^2}{n}}$$

where ME = mean error

d = difference between a specific marker distance on the two films

n = number of marker distances.

The formula gives the root mean square value of differences between marker distances from double determinations at a certain occasion, i.e. the ME is an expression (standard deviation) for the technical accuracy in determining growth. For length error $2 \times n$ was used in the denominator.

Exposure dose measurements

To determine the radiation dose to the skull for each stereophotogrammetric exposure thermoluminescent dosimeters were attached to the top of the cranial vault on two rabbits. Dosimeters were also placed underneath the head to test the exit dose.

RESULTS

Methodological aspects

The technical accuracy is presented in Table I.

Instability (intrasegmental marker distance variation larger than 2

times the technical error), occurred infrequently and was mainly restricted to the nasal bone. This occurred for either a brief time or for the total examination period. Total bone marker loss was rare. In a few rabbits, penetration of the nasal bone into the nasal cavity was observed immediately following the implantation. Balls were also lost through the hole made by the implantation instrument at the site of insertion.

No complete penetration through the endocranial laminar bone into the cranial cavity due to bone remodelling was detected during the observation period.

Dosimeters attached to the top of the rabbit head revealed a maximal exposure dose (51 kV, 16 mAs) of 0.6 mGy. The maximal exit dose obtained was 0.1 mGy.

The frontonasal suture (Fig. 4)

The frontonasal suture was by far the site of fastest bone separation in the rabbit calvarium during the observation period and also demonstrated marker differences in growth between left and right sides. The initial approximate value of 268 $\mu\text{m}/\text{day}$ at age one month decreased in an almost linear fashion, excepting the last 25 days, to about 61 $\mu\text{m}/\text{day}$ at age 21 weeks, or 23% of the initial growth rate. Growth values showed considerable individual fluctuations between growth periods. Subnormal growth rates were noticed followed by a later period of compensatory rebound. A comparison of the right and left frontonasal and coronal sutures in one rabbit is illustrated, demonstrating individual fluctuations and compensatory rebounds (Fig. 5).

The initially registered growth rate value following marker implantation seemed slightly reduced.

The coronal suture (Fig. 6)

The coronal sutural expansion was not as impressive as that of the frontonasal suture. Here, a slightly less pronounced fall in the growth rate was observed. The initial value of about 53 $\mu\text{m}/\text{day}$ declined to nearly

one-fourth thereof, 13 $\mu\text{m}/\text{day}$. The growth-curve fall is not strictly linear and could be described as consisting of two approximately linear phases with a somewhat more rapid initial deceleration. The break in the curve appears about day 70.

Individual differences between right and left sides are not as marked here, even though individual sutural growth fluctuations and compensatory rebounds were observed (Fig. 5).

The sagittal suture (the internasal, interfrontal and interparietal suture; Fig. 7)

Despite fewer evaluations, a distinct pattern of growth deceleration could be detected. The growth in width during this periods was much less pronounced and more constant than that in length. There was also a fall in growth rate along the sagittal suture in the caudal direction. The nasal bones were found to separate with a 2.4 - 3.7 times higher rate than the frontal or parietal bones during the whole observation period. The neurocranial parts of the sagittal suture exhibited initial growth rates of approximately 3% of the frontonasal suture and 17% of the coronal suture. This increased successively to a difference of about 5% of the frontonasal suture and 23% as compared to the coronal suture. Thus, per centual sagittal sutural growth reduction was not as marked as transversal sutural growth reduction. Noted were also infrequent individual negative growth values.

Variations along an ipsilateral transverse suture-line (Fig. 8)

By comparing two pairs of bone markers in 7 animals, the two pairs across the same side (left or right) of the examined transversal suture, minor variations in rate could be detected for the frontonasal suture, but the coronal sutural variations were within the range of technical errors.

Weight control

Regular weight increase was generally observed, and was found inadequate in determining individual animal health. Normal fluctuations in growth could not be distinguished from occasional sickness. As this com-

parison between weight and cranial growth was applied on an individual basis, these results are not considered of presentable value.

DISCUSSION

Experimental procedure

Fundamental to RSA are well-defined anatomic landmarks. Since the rabbit skull lacks any such distinct marks tantalum balls were implanted. A light microscopic study using the same implantation technique disclosed no or only slight fibrosis at the implantation site, and usually only a mild inflammatory reaction in the soft tissues (Aronson and Jonsson, 1976). Alberius (1983 a) demonstrated integration of tantalum markers into bone tissue, and also discussed the processes of marker stabilization and bone remodelling.

Aronson et al. (1977) studied the effect of locally administered unfractionated low dose radiation on rabbit long bone growth with RSA. They found 10 rad (100 mGy) to be without significant growth-retarding effect. Their stereo exposure dose of 0.2 rad (2 mGy) was therefore considered of even less importance. Consequently, our exposure dose of 0.6 mGy (60 mrad; 51 kV, 16 mAs) may be regarded as without influence on somatic growth.

The accuracy (the total technical error) was estimated by complete reappraisals. This amounted to a length error of 14.8 μm and a growth error of 21.0 μm . This error is consonant with the calibration measurements with the same technique on a test rod with tantalum pin markers of known internal distances (Selvik, 1974).

Neurocranial-viscerocranial growth

Both neurocranial and facial sutures were included in the present study. The coronal, interfrontal and interparietal sutures are neurocranial sutures, while the internasal suture belongs to the facial skeleton. The frontonasal suture, at the border between the neurocranial and the facial skeleton, is a craniofacial suture. The location of this suture between the neuro- och viscerocranium suggests an intermediary growth character with a relative dependence on both cranial parts.

Neurocranial growth, guided by neural tissue expansion, is most pronounced perinatally in the rabbit as the brain growth spurt peaks between birth and five days postnatally, and then decreases successively. During the first 60 days about 80% of the adult brain size is attained (Harel et al., 1972). Brain growth causes cranial changes in size and shape by a process of pressure → capsular tension → capsular expansion → reduced tension and so forth (Young, 1959), which is only slightly affected by body growth depression due to nutritional or endocrine etiology (Riesenfeld, 1974). Even at age 5 months, when rabbit body growth is virtually completed (Engdahl, 1972), the cranial vault was still expanding.

A predominance of viscerocranial growth was observed in this study. The internasal suture, in particular, appeared to be influenced by factors other than neural tissue growth, since its growth rates were consistently much greater than the neurocranial part of the sagittal suture. The cranio-facial location of the frontonasal suture implies a closer relation to the neurocranium. However, its break in linear rate deceleration appears at a later point in development than that of the coronal suture, indicating a relative independence from the neurocranium. This dominating facial growth is considered to cause an alteration of skull form from klinorhynchal to orthocranial (Baer, 1954; Moss, 1958; Vilmann and Moss, 1979; Engström et al., 1982).

Sutural growth characteristics

During the observation period the frontonasal suture was found to be the dominating suture for rabbit antero-posterior skull growth. Furthermore, this growth continues for the longest period of time (Ikeda and Honma, 1961). The present report confirms earlier studies concerning a rapid longitudinal linear growth deceleration, but showed higher growth rates compared to most investigations. Thus, Selman and Sarnat (1955), Erickson and Ogilvie (1958), Roskjaer (1977) and Koskinen-Moffett et al. (1981), all following metallic implant separation with cephalometry, and Hong et al. (1968), using vital staining, reported lower rates for various growth periods included in this study. On the other hand, Babler et al. (1982) reported initial marker separation exceeding ours. They found growth,

calculated as a mean rate, to be $393 \mu\text{m/day}$ for day 31 - 60. The most comparable value from Sarnat and Selman (1955), registered between day 42 to 56, is $214 \mu\text{m/day}$. Our values, $259 \mu\text{m/day}$ for day 30 - 60 and $271 \mu\text{m/day}$ for day 46 - 53, consequently are more in accordance with the earlier report.

As for the coronal suture, lesser differences in growth rates have been registered. Recent publications (Persson et al., 1979; Persing et al., 1981; Babler et al., 1982), following rabbits from age 9 days, reported similar initial growth values during day 30 - 60 (recalculated mean rates $73 - 90 \mu\text{m/day}$), markedly exceeding ours. Thereafter, their rates were comparable to what we found. The implantation procedure used has been reported to affect long bone growth only slightly (Aronson and Hansson, 1976). Hence, it can hardly greatly affect passive neurocranial growth. However, stunting of brain growth due to postoperative nutritional deficiency is probable (Alberius, 1983 b), which will affect neurocranial growth. Furthermore, this could explain the presumably reduced initial frontonasal sutural rate value. Nevertheless, as surgical trauma seems comparable using either implant system, the reason for diverging postoperative growth rates can not be explained.

Also, Koskinen-Moffett et al. (1981) registered rate values similar to ours, while the rates found by Hong et al. (1968) and Erickson and Ogilvie (1958) were significantly lower. However, we observed the relative proportion between the frontonasal and the coronal suture to be identical (4-5:1) to Levine (1948), Erickson and Ogilvie (1958) and Koskinen-Moffett et al. (1981).

It is also worth noting that at the frontonasal suture the relative amount of growth of the frontal bone sutural surface is less than that of the nasal bone (Selman and Sarnat, 1955; Ikeda and Honma, 1961; Hong et al., 1968; Koskinen-Moffett et al., 1981). As to the coronal suture, Ikeda and Honma (1961) observed that both bone ends contribute with the same quantitative bone apposition at the sutural surfaces, while Koskinen-Moffett et al. (1981) found dominating frontal bone growth.

The sagittal suture, responsible for the sutural increase in width of the cranial vault, remained open throughout the investigation despite minimal growth, especially caudally. This is contrary to Hoyte (1971) who, using alizarin staining, reported terminated sagittal growth after about 40 days. This is probably due to the low sensitivity of this method.

During the investigation period, sagittal sutural growth is most pronounced in the facial part. We found similar growth values for different segments of the sagittal suture to Roskjaer (1977) and Koskinen-Moffett et al. (1981), and could confirm a relatively uniform pace of separation for the posterior part of the suture. Compared to the frontonasal and coronal suture, the sagittal suture must be considered of minimal significance for cranial vault expansion during the investigated interval.

The observed differences in growth rates might be due to a multitude of factors, e.g. methodological inaccuracy, sexual or strain differences, time and extent of surgical trauma, inexact age-control or varying animal care (nutritional status, litter size). Consequently, to attain representative growth values, our experiment was designed to reduce variability by e.g. using only male rabbits of a well-controlled rabbit strain and strict rules for animal care.

In the present study, two pairs of markers positioned on either side of the sagittal suture demonstrated variations in frontonasal sutural growth rate, most marked during the first half of the experimental period. This confirms earlier reports (Selman and Sarnat, 1955; Yen and Shaw, 1963 a,b; Hong et al., 1968). These variations seem to be a precisely coordinated oscillatory phenomenon, varying from one week to another, yet of little significance since only minor deviations from symmetry could be detected. This demonstrates that bone separation is not a pure translational movement.

A remarkable feature at various examinations of the sagittal suture was occasional negative growth values (maximally $-143\text{ }\mu\text{m}$). These are supposedly caused by elasticity of bone or sutural tissues, or bone resorption. This response was also observed by Hong et al. (1968) and Roskjaer (1977), and might be an integral part of sutural growth (Linge, 1973; Koskinen, 1977),

further indicating that bone separation is not a pure translational movement, but rather an alternating oscillatory separation.

Left-right growth differences of the calvarium (Moss, 1954; Hong et al., 1968; Aaron, 1973; Koskinen, 1977; Sarnat and Selman, 1977) were confirmed, especially pertaining to growth at the frontonasal suture. This suggests the bones to respond individually (but not independently of each other) to normal growth stimuli (Hoyte, 1971). Also, fluctuations in growth rate (Koskinen, 1977) were confirmed. This may be a means of growth compensation by one suture to varied bone separation at another, so called plasticity (Giblin and Alley, 1944). Interestingly, in individual cases, subnormal growth at one suture during one period was regularly followed by a compensatory rebound at the same suture later on. Of note, though, is that the observed left-right growth differences and individual fluctuations of intramembranous bone growth do not seem to be of equal relative importance in long bone endochondral growth (Hansson, 1967; Aronson et al. 1978; Alberius, 1983b).

Hellquist's (1972) statement that rabbits born in summer grew quicker and weighed more than those born in winter was not observed. This influence is probably reduced because of standardized laboratory conditions.

Health control by weight was found inadequate in the present study. Many factors influence the exactness of weight values, like food and water intake as well as excremental losses prior to weighing. Interestingly, Hansson (1967), using the tetracycline method, observed no correlation between weight change and rabbit long bone growth. To obtain a useful health parameter instead of weight, tibial length increase has been tested (Alberius, 1983 b). This parameter is valuable for individual growth registrations, but for averaging a material collected during an extended period of time it is not needed.

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Table I. Statistical accuracy analysis of growth recordings.

marker diameter (mm)	0.8
number of animals	7
number of markers	89
number of distances	529
methodological error (μm)	
growth	21.0
length	14.8

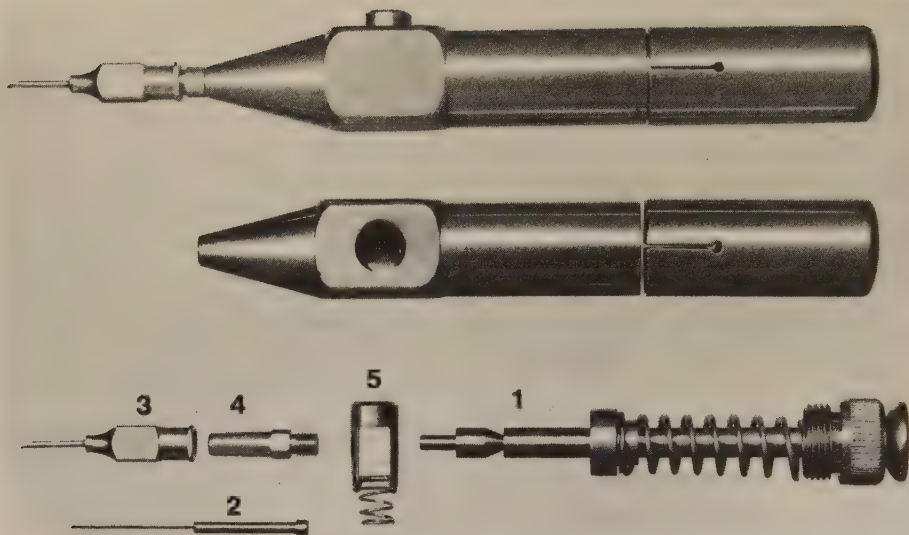


Fig. 1 a Bone marker implant instrument. 1 and 2 = spring loaded piston; 3 = needle; 4 = needle adapter; 5 = trigger mechanism.



Fig. 1 b Connected and disconnected cylindrical stop-block.

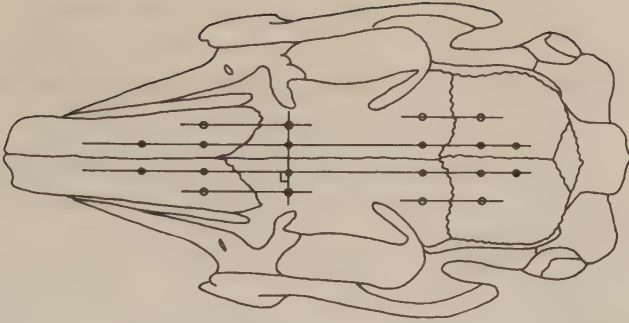


Fig. 2 Tantalum bone marker positioning. The extra markers in 7 animals illustrated (o).

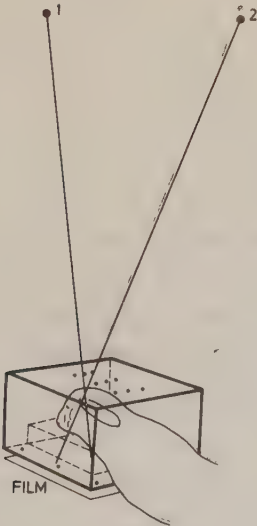


Fig. 3 Roentgen stereo examination. The rabbits head, plated on a support in the calibration cage, was simultaneously radiographed by two roentgen tubes (Focus 1 and 2). Cage reference markers in the upper and the lower plane indicated (-).

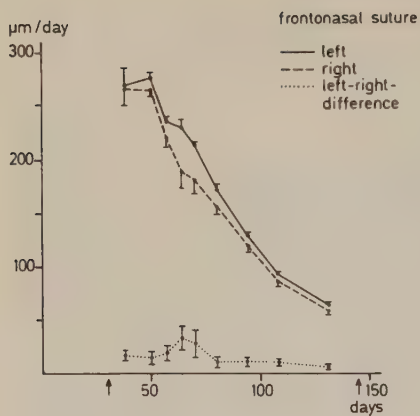


Fig. 4 Frontonasal sutural growth rate. Left: 14 rabbits; right: 13; left-right difference (mean of rate difference in absolute numbers between left and right sides for each animal): 11. The arrows mark start and cessation of the investigation. Brackets indicate the standard error of the mean (SEM).

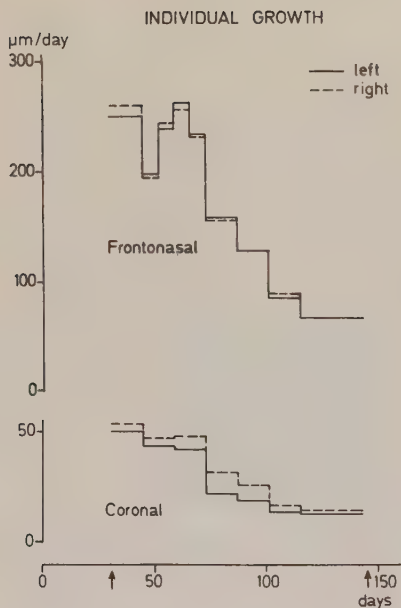


Fig. 5 Frontonasal and coronal sutural growth rates for one rabbit. Note left-right differences, fluctuating growth and compensatory rebound after temporary growth reduction.

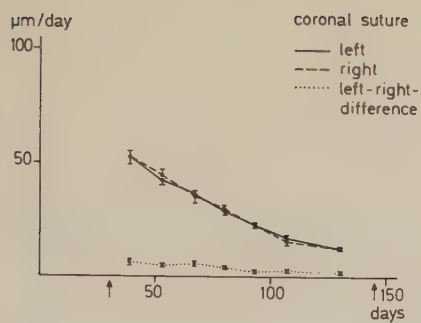


Fig. 6 Growth rate at coronal suture. Left: 16 rabbits; right: 15; left-right difference (see Fig. 4): 15.

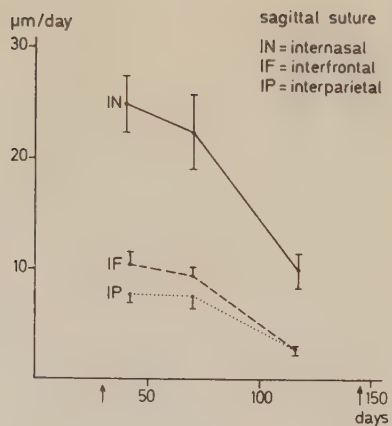


Fig. 7 Sagittal sutural growth rates. Internasal: 11 rabbits; interfrontal: 17; interparietal: 17.

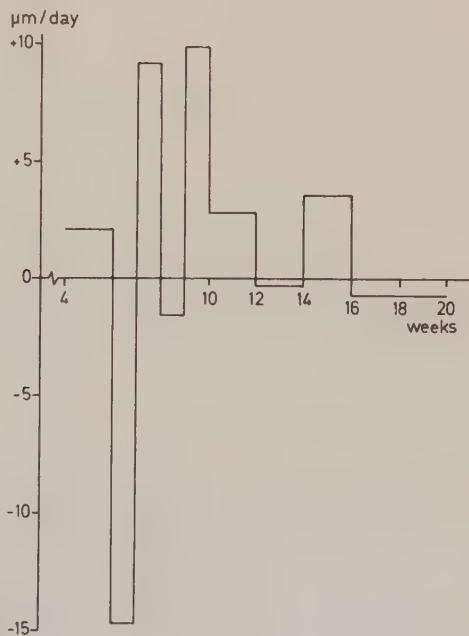


Fig. 8 Comparison of two ipsilaterally positioned pairs of frontonasal sutural markers.

KINEMATICS OF CRANIAL VAULT GROWTH IN RABBITS

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7 Figures, 4 Tables

Running headline: Kinematics of cranial vault growth in rabbits

Key words: bone
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metallic implant
rabbit
roentgen stereophotogrammetry

ABSTRACT

To mathematically characterize the spatial rearrangement of the rabbits cranial vaults bones during growth, a longitudinal study was undertaken from age 4 - 20 weeks. Initially, at least three non-collinear tantalum bone markers were implanted in the parietal, frontal and the combined nasal bones. Thereafter, the animals were followed regularly with roentgen stereophotogrammetrical analysis. The parietal bones were found to rotate laterally upwards (3°), while the frontal bones rotated downwards (2°) relative to their contralaterals. The frontal bones rotated rostrally upwards (12°) and outwards (3°) as well as laterally downwards (5°) in relation to the parietal bones. Due to the morphology of the rabbit head, the examination positioning used in this study and the direction of the growth process, growth at the coronal suture correlated fairly well with longitudinal axis translations, but the growth at the frontonasal suture relative to the frontal bones was directed about 45° downwards. This points to the importance of the bone marker positioning, so that their connecting line is directed along the axis of growth. Also, this approach makes it possible to obtain new information on the development and treatment of craniofacial aberrations.

INTRODUCTION

Calvarial growth proceeds by individual bone changes in size, shape and spatial position. Normally, passive spatial relative relocations, effected by the volumetric expansion of the enclosed neural mass, tend to separate the cranial vaults bones. This results in compensatory marginal bone deposition at the sutures. Furthermore, periosteal deposition, resorption or even stasis participate in skull growth. The quantitative effects of spatial rearrangements, though, are believed to be the dominating growth process (Moss et al., 1972; Moss, 1975).

The decisive role of neurocranial sutures in cranial growth is manifested in premature sutural synostosis. They, besides allowing for spatial adjustments, act as adaptable shock-absorbing soft tissue joints between adjacent bones to prevent any undue separation. Recently, the calvarial suture was also described as a restraining modulating component in cranial growth (Persson, 1973; Persson et al., 1979; Babler et al., 1982; Alberius et al., 1983).

So far, the techniques used for the study of cranial growth e.g. vital staining, radio isotopes and roentgen cephalometry, only permit two-dimensional analysis. Studies have been performed, though, to coordinate assessments of bone growth in different planes. Nevertheless, such analyses are restricted to limited regional sections, making accurate mathematical description of postitional changes impossible. Thus, the demonstration of rotations in all dimensions of a single bone in relation to neighbouring bones has not been possible (Vilman, 1972).

By using roentgen stereophotogrammetry (Selvik, 1974), the kinematics (= the mathematical study of motion, regardless of the cause of the motion) of the cranial vault may be precisely analyzed. By this method movements (rotations and translations) between bones can be determined after marking them with metallic implants. Thus, a longitudinal investigation was carried out to collect information on the spatial changes during growth of the rabbit cranial vault.

MATERIAL AND METHODS

Animals

Seven 4 week old (29.7 ± 0.8 days; 0.729 ± 0.045 kg) male New Zealand white rabbits were used. The animals were kept at $20^{\circ} - 21^{\circ}\text{C}$ (40 % relative humidity), with light from 0600 to 1800 hours, and with standard pellets and water provided ad libitum. The animals were followed until age 20 weeks

(143 ± 0 days) since, according to Engdahl (1972), their growth curve shows a levelling off at an age of five months.

Operative technique

The animals were anaesthetized by intramuscular neuroleptanalgesia (Hypnorm® vet.: Fluanizon and Phentanyl, 10 and 0.2 mg/ml, respectively; 0.6 ml/kg body weight). The head was shaved and the surgical area cleaned with alcoholic Chlorhexidin (5 mg/ml). A para-midline skin incision from the parietal to the nasal region was made. The skin and underlying tissues were reflected laterally and calvarial sutures identified (Fig. 1). Tantalum bone markers (0.8 mm in diameter) were implanted in the nasal, frontal and parietal bones (Fig. 1), using a specially constructed instrument (Aronson et al., 1974). Each bone, referred to as a segment, obtained minimally three non-collinear markers, implanted wide apart to optimize the precision in subsequent computerized calculations. Skeletal morphology was hereby disregarded. Care was taken to minimize periosteal manipulation so as not to influence bone production. After the implantation of bone markers the incision was closed with interrupted 4/0 Ethicon sutures and covered with Nobecutan® spray. The animals were left to recover on a warm electric blanket.

Examination intervals

Initial stereo roentgenograms were obtained on the day of implantation (day 30) and at ages 45, 52, 59, 66, 73, 87, 101, 115 and 143 days.

Roentgen stereophotogrammetric examination

The animals were examined in a glass calibration cage supplied with 0.5 mm tantalum reference markers (Fig. 2). Two simultaneously exposing roentgen tubes were positioned with an approximate focus to focus distance of 0.4 m and a focus to film distance of 0.9 m. The maximal radiation dose used for one examination was 0.6 mGy (Alberius and Selvik, 1982).

Analysis of data

The films were analyzed in a precision instrument for aerial photogrammetry (Wild Autograph A8). The 3-D coordinates of the bone markers were obtained by computer processing. Spatial alterations (rotations about and translations along the cardinal axes) were calculated by a separate computer program (KINEMA) for rigid-body kinematics (see below). A modification of this computer program enabled analysis of translations along the cardinal axes for each implant in relation to a bone chosen as reference (see below). The axes were oriented as follows. The longitudinal axis is situated in the median plane directed anteroposteriorly. The transverse axis is in the horizontal plane, directed at a right angle to the longitudinal axis. The vertical axis is perpendicular to these. Statistical evaluation was conducted; the mean translations and rotations with related standard error of the mean (SEM) are presented.

To facilitate interpretation of movement directions the rabbit head was carefully oriented along the calibration cages longitudinal axis at the initial stereo examination. Subsequent examinations were then automatically reoriented to this position by computer, irrespective of the actual orientation at this particular examination. In two rabbits, though, the initial radiographs disclosed suboptimal skull positions. Therefore, a correctly oriented later examination was selected to mathematically reorient the first one.

Kinematic calculations

Three stable bone markers, at least, constitute a rigid-body model (RBM; Fig. 1) and thereby represent a segment in calculations of motion. Implant stability is checked at each examination by comparing distances and angles between implants to the initial or the preceeding examination. The quality of rigid-body fitting was expressed as the root mean square distance (error) between segment markers in a rigid-body displacement compared to their actual movement. This mean error of rigid-body fitting (e_{rb}) includes both implants deviations from their original positions in the bone

(assumed rigid) and measurement errors. Of note, though, is that a low e_{rb} value does not guarantee high accuracy as markers may be unfavorably positioned, e.g. approximating a straight line. Marker positioning was therefore carefully planned prior to implantation to obtain optimal coverage of the bone.

In evaluations, one RBM was regarded as fixed. At each examination this segment was reoriented to its original position in the laboratory system by computer. Spatial motions between skeletal segments were described as rotations about the three cardinal axes, following classical kinematic principles for rigid-body movement. The computed rotational angles are thus valid for the entire segment regardless of implant localization.

Translations are defined as position changes of points. The segmental translations presented are those of the center of gravity of the implanted markers, thus representing an approximate midpoint of the bone. Translations are given both as their components in the directions of the cardinal axes and as total translation, i.e. the distance calculated from these components using the 3-D Pythagorean theorem. Translations were compared to values obtained from calculations of distance changes between markers in separate bones, i.e. the customary measure of growth. As such values have been presented elsewhere as a function of time (Alberius and Selvik, 1982), only final distance changes are given as a comparison to translation of segments.

In calculating translations of separate implants (point motion), the RBM of the segments were initially tested and the left parietal bone was chosen as reference segment. Thereafter, the 3-D coordinates, and thus translations, of each implant outside this segment were given in comparison to the initial examination. These values were used for computer drawings.

Test of accuracy

To control the accuracy of rotational and translatory measurements, two separate stereo examinations in two different positions were performed on four dried skulls.

Definition

For reasons of simplicity the sutures studied are referred to as calvarial or cranial vault sutures (neurocranium), despite the frontonasal suture being connected to the rabbits facial skeleton (viscerocranium).

RESULTS

Accuracy

Table I illustrates the accuracy of the roentgen stereophotogrammetric method used. The errors represent the standard deviation of rotation angles and translations determined from values obtained when comparing two separately performed stereo examinations on the dried skulls of four animals. This test thus comprises all technical procedures involved, implying that the e_{rb} includes measurement errors as well as technical factors such as film bending. Consequently an e_{rb} exceeding the mean + 2 SD indicates that marker instability (= biological inaccuracy) has occurred, providing a negligible soft tissue mass (= young animals) is present (low roentgen ray scatter).

The rigid-body model

The mean errors of rigid-body fitting (e_{rb}) are presented on an individual basis in Table II. A small deviation from the rigid-body concept is due to a minute movement of an implant in relation to the bone. Thus, the calculated rotations and translations are subject to chance (stochastic) errors, the magnitude of which is impossible to calculate. However, when calculating the movements for a group of animals, these chance errors are included in the dispersion of values around the mean.

Values below 41 μm were considered very satisfactory. Due to frequent marker instability in the nasal bones, these constituted one segment, why growth at the interposed internasal suture implied increasing e_{rb} values

during the investigated interval ranging from 77-210 μm to 758-947 μm .

In two animals, the e_{rb} of the right frontal bone markedly exceeded (280-773 μm and 240-578 μm , respectively) the technical error, wherefore these were excluded from further analyses. Thus, seven animals were utilized for the presentation of interparietal, left fronto-parietal and left naso-frontal movements, but only five were used for the interfrontal and right fronto-parietal movements.

Rotations

The rotations about each of the coordinate axes for the different combinations of bones that have been calculated are presented in Figures 3-5. The resulting effects on relevant bones are indicated in Figure 6.

Transverse axis rotations were not registered between contralateral bones. On the contrary, an increased upward rotation rostrally without any differences between the sides were observed for the frontal bone relative to the parietal bone, finally reaching about 12° .

As described earlier, the two nasal bones together constituted one segment, and could thus only be properly investigated for rotations about the transversal axis. Here a progressive minor flexion relative to the left frontal bone was observed (7.9°). Nevertheless, in comparison to the major extension of the frontal to the parietal bone, an extension of the nasal dorsum relative to the parietal bones was registered.

Longitudinal axis rotations displayed a laterally upward rotation between the parietal bones. On the contrary, the rotations between the two frontal bones were laterally downwards. Finally, the magnitudes of these two rotations were similar, 2.7° and 2.1° , respectively. The opposite rotations described imply that the frontal bones rotate laterally downwards in relation to the parietal bones, obtaining a final mean value close to 5° .

Vertical axis rotations were small and inconsistent, finally displaying an outward movement of the rostral parts, which, however, was significant only for the frontal bones (2.6°).

Translation

Translations were calculated both as total translations and their components along the cardinal axes. Translations, when given for specific points in the bone, may just reflect rotations between the reference and the actual bone, and subsequently does not contribute to further understanding of bone rearrangements. This occurred for transverse axis translations between the frontal and the parietal bones, and longitudinal and vertical axis translations between the parietal as well as between the frontal bones. Furthermore, all these translations were small, finally less than 0.6 mm. However, transverse axis translations between the parietal as well as the frontal bones were considered to represent sagittal sutural growth. On the other hand, longitudinal axis translations between the nasal and frontal bones or frontal and parietal bones were considered to be components of transverse sutural growth.

Transverse axis translations steadily increased parallelly for both the interparietal and the interfrontal sutures, with a final value of 0.77 and 0.74 mm, respectively. Distance changes between markers were found to be 0.71 and 0.76 mm, respectively, for these sutures.

As regards the movements between the frontal and parietal bones (the coronal suture), and the nasal and frontal bones (the frontonasal suture), the situation was more complex. Final values for relevant measures are presented in Tables III and IV, for the coronal and frontonasal sutures, respectively. Besides longitudinal axis translations, the vertical translations, the total translations, the transverse axis rotations and the distance change at the suture is presented for each rabbit. In the coronal suture longitudinal as well as total translations seem fairly well to represent the distance change, exceeding it by 3% and 12 %, respectively.

However, as regards the frontonasal suture, growth does not seem to be directed along the longitudinal axis. Rather, it is represented by the total translation which, when compared to the distance change, only exceeds this by 0.6 %.

For single animals, detailed information on translation of markers projected on the cardinal planes may be obtained by computer drawings. Such a drawing for rabbit No. 7 is reproduced in Figure 7. Longitudinal and transverse growth, as well as rotations about the vertical and transverse axes, are distinctly shown.

DISCUSSION

A new approach to the study of cranial vault growth was used in this study. By the application of roentgen stereophotogrammetry (Selvik, 1974), kinematic growth data (rotations and translations) were registered with high accuracy, as demonstrated by comparison of two separate examinations performed on four skulls. By comparing two means of assessing growth, that is kinematic analysis and distance changes between markers, new aspects on growth dynamics in this region were obtained.

The process of calvarial growth is not merely drift, but also, as demonstrated in this study, centrifugal and longitudinal displacements. Differential sutural growth (giving alternating apposition or resorption at the sutural margin) is considered to be the principle means of producing change of calvarial form (Massler and Schour, 1951; Baer, 1954; Hoyte, 1971; Vilmann, 1972). This process is believed to be directed by neural mass growth, whose direction is influenced by several factors, among which are the degree of flexion of the cranial base and the mode of attachment of dural fiber tracts which underlie the major calvarial suture segments (Moss and Salentijn, 1969; Moss, 1975; Smith and Töndury, 1978; Friede, 1981). Thus, due to the multitude of interrelated factors in calvarial growth, such growth studies require strict definitions of the investigated processes.

Only when growth is directed along a line connecting two markers does a distance increase between the markers represent actual growth. Figure 8, further commented below, illustrates that total translation constitutes the upper limit of a value representing longitudinal growth, while the customary means of measuring distance changes represents the lower value. This is demonstrated for the mean values of both the coronal and fronto-nasal sutures (Tables III & IV). For single animals, deviations from this pattern were only noted at the frontonasal suture in No:s 3 & 7. The only significant case (No. 7) can be explained by asymmetric sutural growth combined with asymmetric location of implants (Fig. 7).

For sagittal sutural growth the transverse axis translations were found to be very close to the value determined from distance changes. This is due to the position of the markers used for the latter registration being close to the suture, with connecting lines parallel to the growth direction which lies along the transverse axis.

However, growth at the two transverse sutures occurred not only along the longitudinal axis, but also comprised a small vertical upward component for the coronal suture and a major downward component for the frontonasal suture. Coronal sutural growth correlated well with longitudinal axis translation, and fairly well with total translation. Rabbit No. 2 illustrates the seemingly paradoxical fact of a distance change significantly less than a component of its translation, explained by Movement 1 in Figure 8. This rabbit showed the largest transverse axis rotation (15.2°) of investigated animals, but the position of the axis of rotation in relation to the implants (not presented), as well as the vertical axis translation (2.0 mm), also influence the relations between translations and distance changes between implants. Thus, in isolated cases, a complex growth process seems to occur, rendering it impossible to determine which measure of sutural growth is optimal when large divergence between the two procedures used is registered.

The frontonasal sutural growth can not be described as a linear process in the direction of the nasal bone since the translations, which are of similar magnitude forwards and downwards, are directed in about 45° to the horizontal plane. That the rotation in the suture plays a less important role is demonstrated by rabbits No:s 5 & 6, which showed an almost pure translation (rotations 3.5° and -1.7° , respectively), but approximately the same proportion between the translations as the other rabbits. The total translation was found to be similar to the distance change, which is explained by Figure 8. In this case the actual movements (nasal bones) are in principle directed as Movement 2, wherefore a good correlation can be supposed.

Transverse axis rotations are the angulation changes observed on lateral radiographs, ordinarily used in roentgen cephalometry. It was unambiguously demonstrated that an upward rotation occurred at the coronal suture (12°). This supports Verwoerd et al. (1979), who determined geometrical changes by standardized photographs of the skull. Estimations with a protractor on their diagram of normal development show a rotation of 17° for the period between age 4 - 20 weeks. At the frontonasal suture, we observed a downward movement (flexion) of 8° , supporting observations by Roskjaer (1977), Persson et al. (1979), Verwoerd et al. (1979) and Persing et al. (1981). Here, the Verwoerd et al. diagram gives 2.5° , while Persson et al. (1979), using roentgen cephalometry, reported 3.6° rotation between days 30 - 90.

Thus, we obtained a final quotient of 1.5 between the coronal and frontonasal sutural rotations. This value (> 1) signifies an orthocranialization (e.g. Moss, 1958; Pucciarelli, 1981, and Engström et al., 1982), giving a change from the globular neurocranium of the newborn to the more dolicocephalic skull of the older animal (e.g. Baer, 1954, and Hoyte, 1971).

Longitudinal axis rotations are the angulation changes observed in cross-sections of the skull. The opposite, but in magnitude equally large rotations between the parietal and frontal bones, respectively, have not been,

to our knowledge, described earlier in the rabbit. As the rotations are in opposite directions, torsional forces are developed in the coronal suture. This observation furthermore supports earlier theories that the brain does not expand uniformly in all directions.

Vertical axis rotations imply that the growth rate across the sagittal suture is unequal rostrally and caudally. The present investigation showed a final outward rotation of the rostral portion of the frontal bones during the interval 30 - 143 days, which, assuming symmetric growth, was 2.6° for each bone. Thus, for a typical intra-bone marker distance of 10 mm, the registered sutural growth at the anterior marker pair is $10 \times 5.2^{\circ} \times 0.0175^{1)} \text{ mm} \cong 0.9 \text{ mm}$ larger than at the posterior pair. This major difference (0.9 mm), exceeding the calculated sagittal sutural bone separation (0.8 and 0.7 mm for the parietal and frontal bones, respectively), implies that a marker pair initially situated in the middle of the bone must be used to obtain representative growth values.

In conclusion, this study points to the fact that spatial rearrangement at calvarial sutures occurs up to maturity, implying that the shaping of the cranial vault is a continuous process even during minimal brain growth (Harel et al., 1972). Furthermore, roentgen stereophotogrammetry may give new aspects on the kinematics of the development of experimental cranial aberrations.

¹⁾ Conversion factor from angular degrees to radians.

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Table I. Methodological accuracy testing. The formula:

$$SD = \sqrt{\frac{(\text{Movement } 1 \rightarrow 2)^2}{n}}$$

was used. Movement 1 → 2 signifies the rotation or translation between experiments 1 and 2 for a certain axis, and n = segment combinations.

Number of animals	4	
Number of markers (0.8 mm)	88 (18 segments)	
Number of segment combinations	11	
Methodological error, rotation	transverse axis	0.25°
	longitudinal axis	0.43°
	vertical axis	0.16°
	translation	
	transverse axis	50 μm
	longitudinal axis	26 μm
	vertical axis	84 μm
Mean error of rigid-body fitting (e _{rb})	22.2 ± 9.4 μm	

Table II. Errors of rigid-body fitting (e_{rb}) for the parietal and frontal bones (in μm).

Animals	Observations								
	1-2	1-3	1-4	1-5	1-6	1-7	1-8	1-9	1-10
Left parietal bone									
1	39	47	48	35	49	43	53	37	58
2	35	27	23	26	44	26	42	53	59
3	15	13	12	4	20	19	12	19	32
4	30	23	29	33	29	27	30	26	38
5	5	12	22	14	22	15	41	51	72
6	11	19	28	12	23	40	40	59	37
7	16	11	20	14	20	9	17	21	62
Right parietal bone									
1	13	12	18	13	31	30	26	26	30
2	23	27	31	32	26	24	30	37	44
3	19	55	46	48	81	74	79	116	162
4	11	10	15	8	26	13	20	15	34
5	36	51	24	43	45	51	69	83	102
6	35	27	38	66	27	48	49	73	92
7	16	15	11	19	17	50	85	99	143
Left frontal bone									
1	70	64	81	71	40	78	125	104	102
2	61	33	15	31	34	49	11	37	13
3	32	22	18	28	45	16	16	33	36
4	44	11	65	16	29	29	53	35	100
5	46	73	63	140	144	157	157	165	210
6	23	19	9	49	21	16	28	23	32
7	67	31	26	44	18	42	38	71	105
Right frontal bone									
1	21	25	21	30	31	38	73	101	163
2	56	37	13	55	41	36	29	45	59
3	127	69	95	100	117	129	144	145	172
4	29	42	36	18	22	59	45	53	116
5	12	18	21	13	20	41	49	124	109

Table III. Movements registered between the frontal and the parietal bones (means of right and left sides). In degrees and mm.

Animal	Rotations	Translations		Total	Ordinary growth registration
	Transverse axis	Longitudinal axis	Vertical axis		
1	9.40	2.602	0.475	2.699	2.696
2	15.18	3.862	2.035	4.424	3.247
3	12.58	2.794	0.970	3.051	2.743
4	12.43	3.431	1.665	3.833	3.210
5	9.04	2.636	1.007	2.822	2.746
6	13.60	3.013	1.335	3.306	3.285
7	11.61	3.114	0.731	3.256	2.912
$\bar{m}$	11.98	3.06	1.17	3.34	2.98
SEM	0.83	0.17	0.20	0.23	0.10

Table IV. Movements between the nasal and the left frontal bones (in degrees and mm). Upwards + ; downwards - .

Animal	Rotations	Translations		Total	Ordinary growth registration
	Transverse axis	Longitudinal axis	Vertical axis		
1	- 5.29	10.092	- 15.470	18.563	18.148
2	- 19.24	9.771	- 16.038	18.840	18.563
3	- 9.05	10.483	- 12.993	17.083	17.170
4	- 16.38	12.930	- 11.796	17.555	17.307
5	3.48	14.864	- 8.204	17.057	16.624
6	- 1.66	9.599	- 13.634	17.190	17.083
7	- 6.98	12.274	- 11.055	16.584	17.171
$\bar{m}$	- 7.87	11.43	- 12.74	17.55	17.44
SEM	3.00	0.75	1.02	0.32	0.18

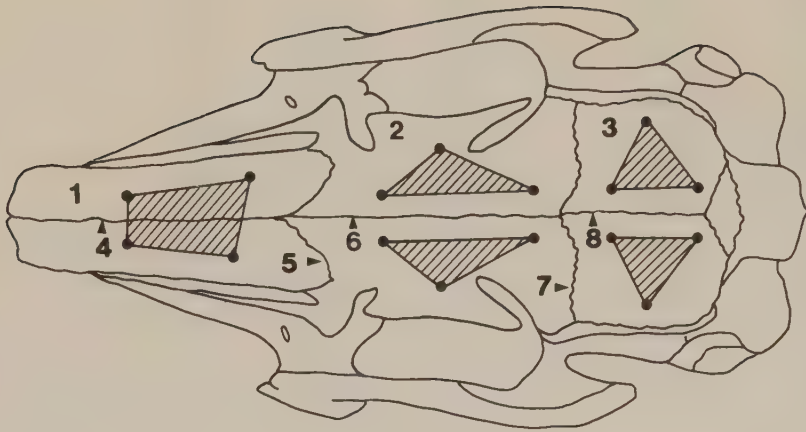


Fig. 1 The anatomy, bone marker (•) positioning and the rigid-body models (RBM; stippled areas) studied.

Bones: 1. Nasal, 2. Frontal and 3. Parietal.

Sutures: 4. Internasal, 5. Frontonasal, 6. Interfrontal, 7. Coronal and 8. Interparietal.

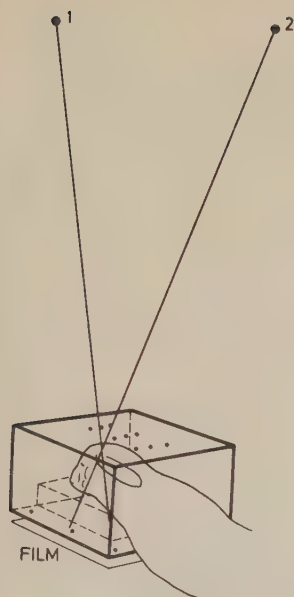


Fig. 2 Roentgen stereo examination. The rabbit head is placed on a support in the calibration cage and is simultaneously radiographed by two roentgen tubes (Focus 1 and 2). Cage reference markers in the upper and lower plane indicated (•).

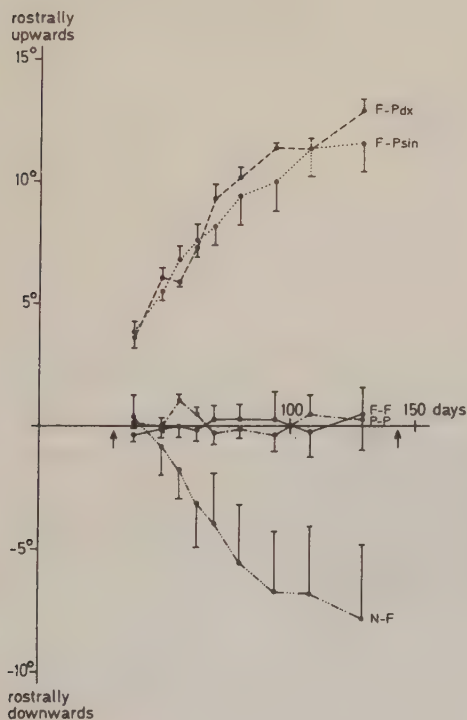


Fig. 3 Rotations about the transverse axis. P = parietal bone; F = frontal bone; N = nasal bones. Arrows represent start and cessation of investigation period. Brackets indicate the standard error of the mean (SEM).

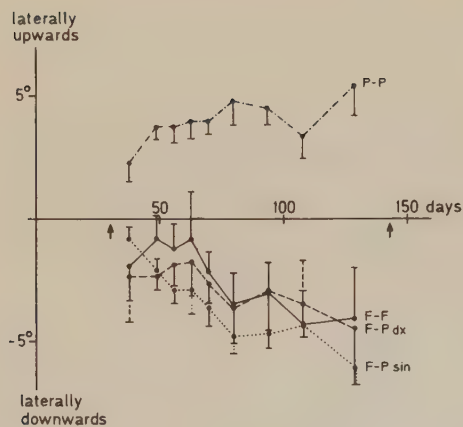


Fig. 4 Rotations about the longitudinal axis (see Fig.3).

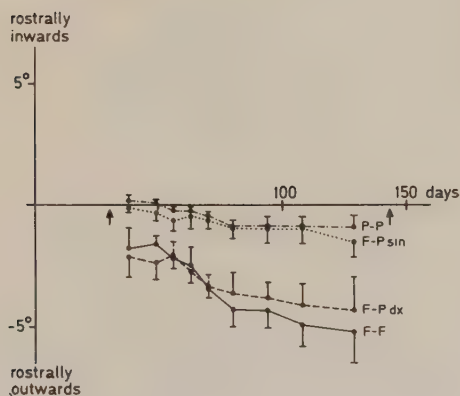


Fig. 5 Rotations about the vertical axis (see Fig. 3).

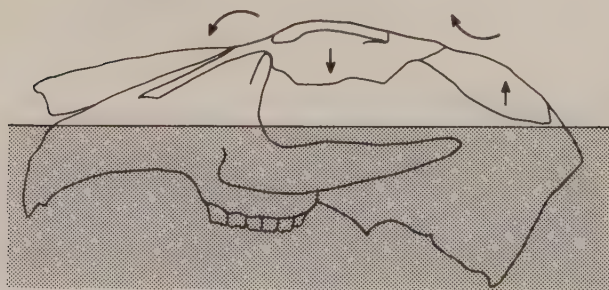


Fig. 6 Illustration of calvarial bones rotations. Arrows signify transversal ($\curvearrowright$) and longitudinal ($\updownarrow$) axes rotations.

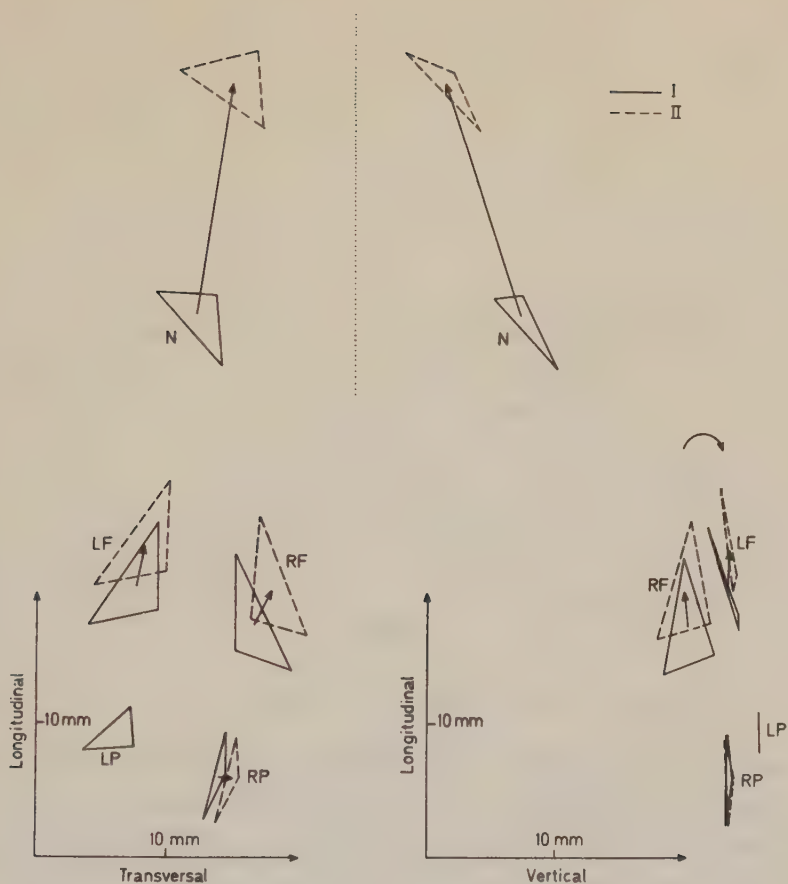


Fig. 7 Movements of calvarial bones in rabbit No. 7 represented by marker displacements relative to the left parietal bone. The left frontal bone rotation was 9.4° and the right frontal bone 7.2° , while the nasal bones showed no rotation to the reference bone in this particular case. Arrows ($\uparrow$) indicate the displacement of the center of gravity of the marker triangles in the respective bones. Arrow ($\curvearrowright$) signifies rotation of a segment. The skull seen from above (longitudinal and transverse axes) and from the left side (longitudinal and vertical axes). P = parietal bone; F = frontal bone; N = nasal bones; L = left; R = right. Initial (I) and final (II) examination.

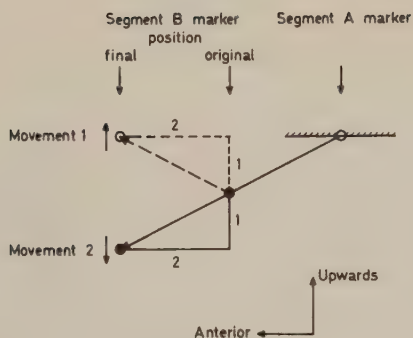


Fig. 8 Movement 1 and 2 exhibit the same total translation = $\sqrt{5}$. Distance change of Movement 1: $4 - \sqrt{5} \approx 1.8$, and of Movement 2: $\sqrt{5} \approx 2.2$. A registered length change never exceeds the marker translation, but will, however, be identical to this value when the connecting line describing the length change coincides with its translation vector. Also, the length change may be less than any component of its translation, illustrated here by the anterior component of Movement 1.

ROENTGEN STEREOPHOTOGRAMMETRIC ANALYSIS OF NEUROCRANIAL SUTURECTOMY
IN RABBITS

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grammetry, Skull growth, Suturectomy

SUMMARY

This investigation was conducted to further elucidate both the significance of a calvarial suture and the cranial vault compensatory ability. Four week old male New Zealand white rabbits were subjected to uni- or bilateral extirpation of the coronal suture after insertion of metallic markers and subsequently followed regularly by roentgen stereophotogrammetry until age 21 weeks. Bilateral extirpation of the normal coronal suture resulted in a dramatically increased initial bone separation rate which tended to remain supranormal for the rest of the investigation. Unilateral suturectomy showed differences in growth between the sides, the operated side initially separating significantly more. Volumetric calvarial growth in unilateral extirpation terminated similar to controls, while volume in bilateral extirpations constantly exceeded controls, finally exceeding these by 65%. Responses at intact sutures confirmed the cranial vaults compensatory capacity. The results indicate that the passive longitudinal and volumetric cranial vault bone growth responds quickly to growth disturbances, thereby demonstrating its plasticity, and that the neurocranial suture is a restraining-modulating component in cranial growth.

INTRODUCTION

Advances in cranial surgery have stimulated interest in the nature of neurocranial sutures. These extremely adaptable fibrous articulations allow bone deposition in response to neural expansion during pre- and postnatal growth. The effect of prematurely terminated bone separation at the calvarial suture, whether primary or secondary, is clearly demonstrable in craniosynostosis, resulting in abnormal skull shapes. By early surgical re-creation of sutures, cranial form is often improved dramatically.

Studies to gain new knowledge of sutural function have been performed by many means, one being extirpation of sutures. This operation has been utilized to confirm whether sutural location is predetermined or not (14, 26,43,44), to study the growth potential of sutural tissue (11,12,17,36,

38,47), sutural morphology (27,32,44), dural bone regeneration (20,21,29,43), periosteal regenerative capacity (35) and embryonic sutural behavior (23). Much attention has also been focused on sutural influences on cranio-facial development. Removal of various facial and neurocranial sutures has given diverging experimental results with no (10,18,19,26,39,40,44-47) or slight (6,7,13,31,35) growth disturbances.

These conflicting views call for new investigations performed with highly accurate techniques to elucidate the function of and interactions between sutures. This information is essential to optimize the extent of surgical intervention in cases of sutural disturbance. The purpose of this investigation was therefore to biometrically clarify the effects of the absence of a neurocranial vault suture, per se and on adjacent intact calvarial sutures, as well as on calvarial volumetric changes, in the hope of improving the design of neurocranial operations.

MATERIAL AND METHODS

Animals

Twenty (10 controls and 10 experimental) approximately 4 weeks old (32 ± 2 days) male New Zealand white rabbits were used. The animals were kept at $+20^{\circ}\text{C}$ (40% relative humidity) with light from 6 a.m. to 6 p.m.. Standard pellets and water were provided ad libitum. The rabbits were followed until 21 weeks (146 ± 7 days) since, according to Engdahl (10), a levelling off in their growth curve is shown at an age of 5 months.

Operative techniques

The animals were anaesthetized by intramuscular neuroleptanalgesia, Fluanizon and Phentanyl, 10 and 0.2 mg/ml, respectively; 0.6 ml/kg body weight (Hypnorm[®] vet.). The rabbit head was shaved, after which an antiseptic solution (alcoholic Chlorhexidin, 5 mg/ml) was applied to the surgical site. The experimental site was exposed by a para-midline incision from the parietal to the nasal region. The scalp and underlying tissues were reflected laterally and the calvarial sutures identified (Fig. 1). Spherical tantalum bone markers were implanted as illustrated

in Fig. 2. Thereafter, 7 mm of periosteum on each side of the coronal suture was extirpated and the suture, including 5 mm of adjacently located parietal and frontal bone, was removed with a burr mounted on a dental drill.

Six bilateral coronal suturectomies stretching to and including the adjacent part of the temporal bone (31) and 4 unilateral extirpations (left side), extending slightly across the midline, were made (Fig. 3). Extreme care was taken to avoid damaging the underlying dura. Bone wax was fixed to the bone edges and hemostasis secured. Zenkers modified solution (without glacial acetic acid) was applied to the dura with cotton swabs for one minute, avoiding the superior sagittal sinus, to retard bone regeneration (5). After copious irrigation of the surgical field with sterile saline, Nebocetin[®] antibiotic powder (Neomycin 0.33 g, Bacitracin 25.000 IU) was sprinkled over the wound. The incision was closed with 4/0 Ethicon sutures and the surgical site finally covered with Nobecutan[®] spray. The animals were left to recover on a warm electric blanket. The postoperative period was uneventful.

Roentgen stereophotogrammetric analysis (RSA)

A RSA-method has been developed to enable precise skeletal measurements in patients or experimental animals. This method requires the implantation of metallic markers and certain roentgen calibration equipment in which the subject is stereometrically examined (Fig. 4). The equipment (a glass cage with markers of known internal positions, lying in two parallel planes), defines a 3-D laboratory coordinate system, enabling determination of the subjects markers as derived by computer calculations based on measurements from the exposed films. The two simultaneously exposing roentgen tubes were positioned with an approximate focus to focus distance of 0.4 m and a focus to film distance of 1.0 m. Exposure data for a rabbit cranial vault examination were 48-51 kV and 4-16 mAs, depending on age.

Methodological accuracy has previously been determined (2). Complete re-examinations of differently positioned rabbit skulls were performed, exhibiting a technical error of 21.0 μ m for growth measurements.

Correspondingly, an error of 0.48% has been obtained for volumetric calculations.

Stereoroentgenograms were obtained on the day of implantation and at weekly intervals until age 10 weeks, thereafter at weeks 12, 14, 16 and 21. Weight control was carried out at each examination.

Analysis of data

The films were digitized in a precision instrument for aerial photogrammetry (Wild Autograph A8). The 3-D coordinates of the bone markers were obtained by computer processing and further analyzed by computer to obtain volumetric as well as distance changes. Volume was calculated from a polyhedron with corners at all markers excepting the nasal bone markers, constructed as the closest to convex polyhedron in a specific sense (9) from the given marker configuration. Growth rates (means of left and right sides) were related to a mean day in the investigation interval and expressed in $\mu\text{m/day}$; volume changes were given in per cent of the initial volume.

To reduce the influence of technical errors (see below) on growth rate values, the coronal suture was observed for minimally two-week intervals. The sagittal suture, whose growth between succeeding measurements approached the technical error, was analyzed only for weeks 4, 7, 12 and 21.

The variations in numbers of animals in each analysis were due to non-optimal implantation procedures, which in some cases resulted in marker losses or oblique localization across a suture (2), hence a few analyses were impossible to undertake. Also, some rate values were excluded to avoid influence from unrepresentative growth values, e.g. due to major illness (as determined by weight loss).

For reasons of simplicity the sutures studied are referred to as calvarial or cranial vault sutures, despite both the internasal and frontonasal sutures being connected to the rabbits facial skeleton.

Statistical analysis was performed using the Student t-test, the results being presented in the diagrams. Since no specific hypothesis of differences between experimental groups was tested, only comparisons of the experimental groups versus controls were considered.

Microscopic investigation

At the termination of the experimental period the degree of bone regeneration was controlled post-mortem under a dissecting microscope (Zeiss, Opton).

Using a protractor, dorsal radiographs were analyzed to determine whether snout deviation from the neurocranial midline was present. Any deviations were recorded to the nearest one-half of a degree in absolute figures.

Mean total longitudinal growth

To compare growth at different sutures the mean total sutural growth values of the total investigation period are given.

RESULTS

Macroscopic findings

The originally wide coronal sutural extirpations remained mostly unossified. In all animals, these were covered by dense fibrous tissue without observable bony islands. Generally, unilaterally resected rabbits reossified from the extirpated margins to a greater degree than bilaterally extirpated, especially adjacent to the temporal bone. The extirpation defects were bordered by protruding bone spicules, their long axes mainly oriented along the antero-posterior axis of the vault, the parietal and frontal bones appearing to react similarly. Overall morphology of unoperated parts of the relevant bones and sutures were comparable to controls. The bone tissue seemed unaffected by the presence of tantalum markers and bone covering the markers appeared similar to adjacent areas. Marker loss was rare, and almost completely restricted to the nasal bones.

Lateral deviation of the snouts (unilateral extirpation: range 1.0°)

to 2.0° , mean 1.6° ; bilateral extirpation: range 1.0° to 1.5° ; mean 1.1°) were similar to controls (range: 0° to 2.0° ; mean 1.1°).

The frontonasal suture (Fig. 5)

A pattern of rapid, almost linear, growth deceleration was observed. The frontonasal suture was the site of fastest bone separation in the rabbit calvarium during the observation period. Initially, bone separation tended to slightly exceed controls in bilaterally extirpated animals, while unilaterally extirpated rabbits showed somewhat subnormal rate values. Contrary to controls, the fall in growth rate for bilaterally suturectomized animals tapered off during the last part of the investigation.

The coronal suture (Fig. 6)

Generally, antero-posterior growth at the coronal suture was less pronounced during the experimental period as compared to the frontonasal suture. Throughout the greater part of the investigation, bilaterally extirpated animals showed marker separation to significantly exceed controls and unilaterally extirpated animals, excepting the final period when both uni- and bilateral suturectomy resulted in marker separation markedly exceeding controls. Otherwise, unilaterally extirpated animals growth rates nearly paralleled control values, the extirpated side initially exceeding the unoperated.

The sagittal suture (the internasal, interfrontal and interparietal suture) (Fig. 7)

Despite fewer evaluations, a distinct pattern of growth deceleration could be detected. The nasal bones were found to separate with a 1.9 to 5.9 times higher rate than the frontal or parietal bones during the whole observation period.

Nevertheless, growth rates, and hence the shape of the curves of the 3 sections, were similar. Only a significantly reduced initial interfrontal

sutural growth rate was observed following unilateral extirpation. Thus, coronal suturectomy only slightly changed sagittal sutural growth.

Volume (Fig. 8)

The neurocranial (polyhedron) volume of control animals showed a tendency to increase slowly. A slight fluctuation was observed for the different intervals during the whole observation period, ranging from 1.8 to 4.2% of the preceeding volume. A minor relative increase was observed during the last part of the investigation. The expected levelling off in brain growth was apparently not followed by a reduction in the calvarial volumetric increase during the span of this investigation.

A similar, initially elevated, volumetric increase was observed for both experimental groups. Thereafter, bilateral extirpation produced a constantly elevated near-parallel increase compared to controls, finally rising beyond controls by approximately 65%. On the other hand, expansion following unilateral extirpation terminally conformed to controls. The irregularity of the control curves were duplicated in the experimental groups. However, in bilaterally extirpated animals, neurocranial volume was observed to reach a plateau.

Mean total longitudinal growth (Table I)

Bone separation at the coronal suture was significantly increased by bilateral extirpation, as was the cranial vault width. Unilateral suturectomy resulted in only minor deviations, and sagittal sutural growth in both groups was not significantly affected.

DISCUSSION

Bone regrowth

To delay osseous regrowth in the extirpation defect and thereby minimize interference with bone separation the calvarial periosteum (dura mater and pericranium) was manipulated. The outer periosteum was excised about 2 mm distant around the suturectomy, while modified Zenkers solution (glacial acetic acid excluded) was applied to the dura. Anderson and Johnson (5)

first introduced this tissue fixative in treatment of craniosynostosis in order to minimize the risk of premature resynostosis.

Today there is little doubt that not merely cells of the outer calvarial periosteum but also those of the dura exhibit osteogenic potential. The importance of the dura during normal development and bone redevelopment has been confirmed by many experimental investigations (14,20,21,26,29,33,34,42,43,48) and various clinical reports on craniosynostosis (e.g. 5,22,41).

There has always been concern that the dural application of Zenkers solution might damage the underlying cerebral cortex. This has now been experimentally verified in the cat and dog (24,25). In man this manifests itself by seizures or neurological deficiencies. By using the modified Zenkers solution, a smaller volume of solution, an application time reduced to one minute, and careful avoidance of the superior sagittal sinus, all followed by extremely careful rinsing, the risk of cortical damage is presumably reduced. Application of this modified technique has been found safe and valuable, giving a minimal recurrence of sutural refusion after surgical intervention even in scaphocephalic children (Alberius, Brandt and Selvik, unpublished data 1983). Furthermore, no obvious behavioral changes were observed in the experimental animals.

Longitudinal cranial vault growth

When studying specific sutural reactions after suturectomy, adhesion to adjacent tissues and structures that may interfere with succeeding bone separation must be considered. As to the rabbits frontonasal suture, Roskjaer (37), Freng (13) and Babler et al. (6) comment on the results of Selman and Sarnat (40), who extirpated this suture without any observed changes in growth. Roskjaer (37) explained this as due to maintained contact between the frontal and nasal bones through the ethmoturbinates, while Freng (13) supposed the unaffected growth to depend on a strong sutural connection to the maxilla. Babler et al. (6), on the other hand, considered Selman and Sarnats (40) animals too old. Additionally, they remarked that while the coronal suture primarily responds to the

increasing brain mass, the frontonasal suture is also influenced by mid-facial growth. Thus, to minimize interference on the experimental area, the coronal suture was selected in the present study. This suture, when growing, provides the advantages of being influenced mainly by one functional matrix (28), and no restricting tissue unions seem to exist. Suture function could thereby be more accurately investigated.

After bilateral coronal suturectomy, the frontal and parietal bone separation showed a significantly accelerated initial pace, followed by an increased rate for the most part of this investigation, as compared to controls. This agrees with some previous observations (6,7,31). These, however, exhibited generally higher growth values. These values can partly be explained by lacking compensation for geometric magnification; also intrastrain and sex differences should be considered.

Since complete removal of the coronal suture entailed bilateral removal of the adjacent parts of the temporal bone, lateral restrictions in respect to the frontal and parietal bones were eliminated. This elimination was manifested by increased cranial vault width. Changes of symmetry, on the other hand, were not observed. The average nasal deflection obtained was identical to our controls, and similar to others, e.g. Alhopuro (4) (1.3°) and Bardach et al. (8) (0.98°).

The highly accurate RSA method also enabled the detection of increased bone separation on the extirpated side immediately after unilateral suturectomy, accompanied by a slightly increased snout deviation.

Presumably, these results express a liberation of biomechanical stresses due to neural mass growth. Thus, the suture tissue proper is a restraining component during normal growth (6,30,31), having a modulating effect on skull growth (6,31). Furthermore, this supports the passive nature of cranial vault growth, pointing to the importance of full surgical release of fused sutures (6), and an exact positioning of surgically created sutures in cases of synostosis so as to obtain optimal postoperative cosmetic results.

So far, only Levine (18), Persson et al. (31) and Babler et al. (6,7) have investigated removal of the normal rabbit coronal suture. Only Babler et al. (6,7) reported on subsequent sutural interaction in parts of the rabbit cranial vault. Disturbance of normal skull growth is known to alter growth not only at the site of disturbance but also at adjacent "normal" sutures (6,7,16). We found remaining intact calvarial sutures showed somewhat different behavior, in particular following bilateral suturectomy. The initial decrease of interfrontal sutural growth in unilaterally extirpated animals demonstrated that even slow-growing vault sutures are sensitive sites of compensation. Macroscopically, though, skull morphology remained similar to normal, indicating other sutural areas might have adjusted to the observed local overgrowth (31).

By its changes in growth rate, the frontonasal suture seems connected to neural mass expansion. It can be speculated that the registered changes in calvarial growth pattern are due to spatial rearrangements of cranial bones (6,7,31) which redirect brain growth, in combination with a larger capacity for calvarial adjustment due to its association with the later maturing facial structures (31). Interestingly, Babler et al. (6,7), contrary to our results, found bilateral suturectomy to cause reduced growth at the frontonasal suture. We, however, noticed an initial stimulation of growth. These diverging results may be explained by variations in animal health. This is to be further discussed in a companion paper (1).

It has recently been suggested that the responsive variations to diverse influences at various sutures may reflect suture-specific differences in reaction to disturbances of normal growth relationships (6). Considering the divergent responses observed between our experimental groups, this investigation rather supports that vectorial changes in neural tissue expansion (28) regulate the individual suture response.

Volumetric growth

This is the first time the volumetric growth of a representation of the calvarium has been followed with high accuracy. To obtain a volume as representative as possible for cerebral volume, markers were introduced into

the temporal bones. The nasal bone markers were subsequently excluded from the calculations. In controls, the volumetric growth rate fluctuated continuously without any tendency to diminish during the observation period. The lack of initial volumetric increase may be attributed to the surgical trauma at bone marker implantation.

Both suturectomized groups showed a marked initial volumetric increase, presumably an effect of decreased sutural restraint. Following an irregular course, growth in unilaterally suturectomized animals culminated in a final volume similar to controls. On the other hand, a dramatic near linear volumetric increase was observed following bilateral extirpation. This paralleled controls and finally reached a plateau, which was not observed in controls during the same period. However, it can not be excluded that this increase in calculated volumes might be partly due to changed external morphology, giving a higher calculated polyhedral volume without a corresponding brain case increase. This is plausible as neural tissue growth is considered to be almost completed during the period ending this investigation (15). Subsequently, as the intracranial volume is presumably increasing only minimally, a rearrangement of neurocranial bones may thus be taking place. This would imply a rotation of the cranial vault bones. By kinematic analysis, continuous rotations of rabbit calvarial bones were demonstrated during this interval, despite the successive diminution of longitudinal growth (3).

This may indicate that bilaterally extirpated animals sooner reached the expected final spatial bone arrangement after liberation of expansive forces. This suggests spatial adjustments were more easily accomplished. This further supports Perssons (30) theory concerning the restricting capacity of structures associated with a suture. Consequently, this strongly emphasizes a careful planning of cranial surgery involving sutural manipulation during infancy, in order to avoid interference with normal neurocranial development due to excessive liberation of sutural restricting forces.

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TABLE I. Mean longitudinal growth rates per day. Significance levels (Student's t-test) indicated:

* p ≤ 0.05
** p ≤ 0.01
*** p ≤ 0.001

	<u>E_x_t_r_a_p_a_t_i_o_n</u>						<u>C_o_n_t_r_o_l_s</u>		
	<u>u_n_i_l_a_t_e_r_a_l</u>			<u>b_i_l_a_t_e_r_a_l</u>					
	$\mu\text{m/day}$	SEM	n	$\mu\text{m/day}$	SEM	n	$\mu\text{m/day}$	SEM	n
Internasal suture	16.6	1.5	2	13.1	1.8	4	20.6	3.3	6
Frontonasal suture	116.2	9.4	6	164.6	6.2	9	142.3	11.1	17
Interfrontal suture	6.1	0.7	4	4.9	1.8	6	6.5	0.6	10
Coronal suture	28.3	2.3	7	41.6***	2.0	12	28.4	0.8	20
Interparietal suture	3.3	1.8	3	5.3	1.2	6	5.3	0.7	10
Cranial vault width	17.1	4.8	3	29.0*	3.0	5	19.3	2.2	7

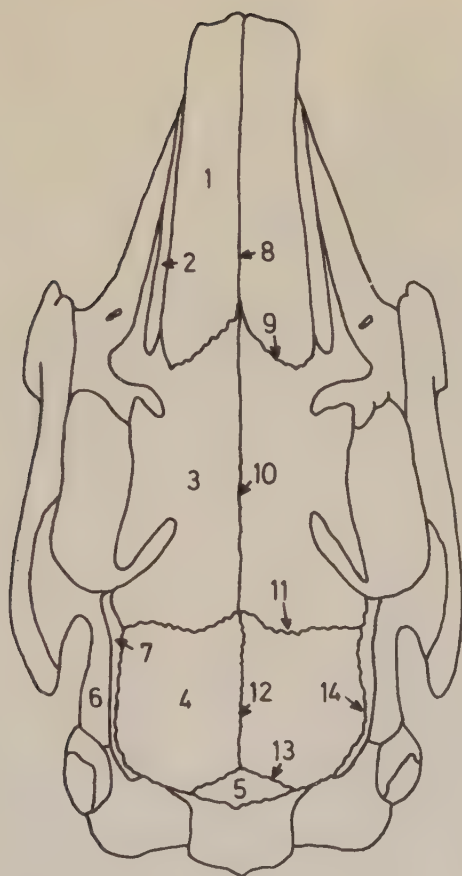


Fig. 1 Relevant anatomy. Bones: 1. Nasal, 2. Premaxilla, 3. Frontal, 4. Parietal, 5. Interparietal and 6. Temporal. 7. Temporal line. Sutures: 8. Internasal, 9. Frontonasal, 10. Interfrontal, 11. Coronal, 12. Interparietal, 13. Lambdoidal and 14. Temporal.

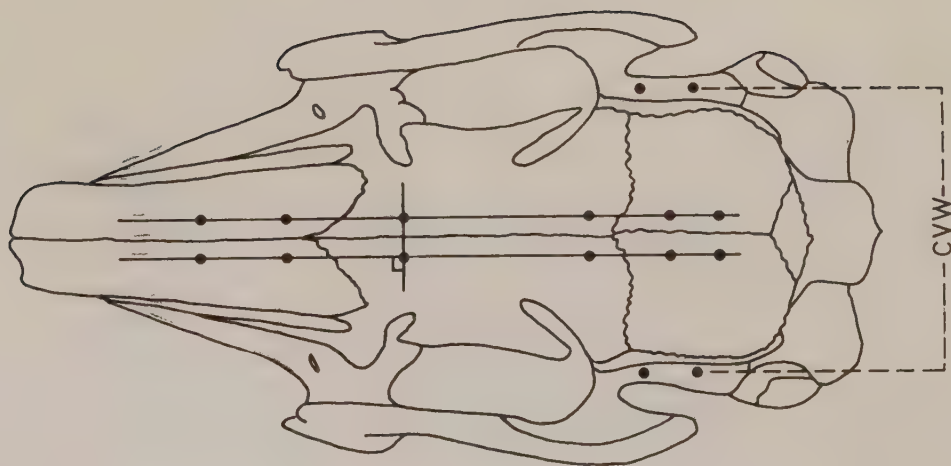


Fig. 2 Tantalum bone marker positioning. CVW signifies cranial vault width.

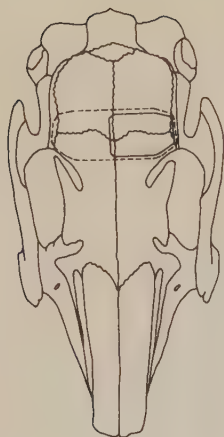


Fig. 3 Dorsal view of rabbit skull illustrating the design and extent of bilateral (- - -) and unilateral (-----) sutural extirpation.

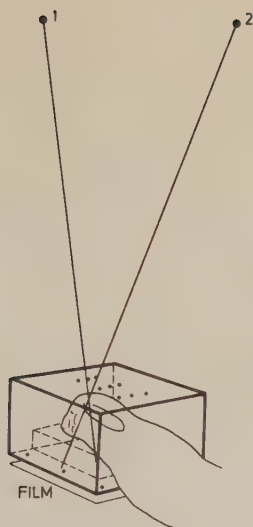


Fig. 4 Roentgen stereo examination. The rabbit head is placed on a support in the calibration cage and is simultaneously radiographed by two roentgen tubes (Focus 1 and 2). Cage reference markers in the upper and the lower plane indicated (•).

FRONTONASAL SUTURE

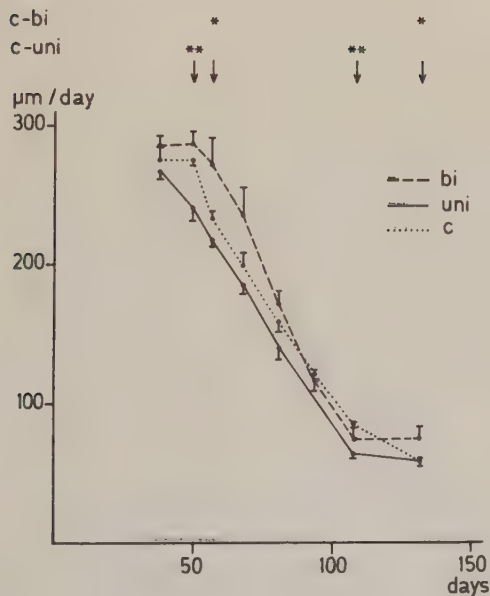


Fig. 5 Frontonasal sutural growth rates. Bi = bilateral coronal suturectomy (n=10; two sides with non-optimal marker implantation excluded), uni = unilateral (n=4; extirpation side), c = controls (n=17). Standard error of the mean (SEM) indicated with brackets. Significance levels (Student t-test) indicated: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

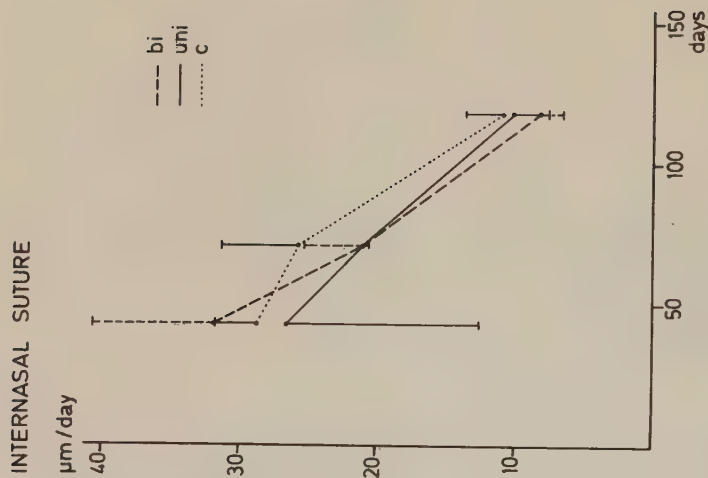


Fig. 7 Growth rates of sagittal sutures. Internasal, bilateral n=5 (rabbits), unilateral n=2, controls n=6. Interfrontal, bilateral n=6, unilateral n=4, controls n=10. Interparietal, bilateral n=6, unilateral n=3, controls n=10. Abbreviations and significance levels in Fig.5.

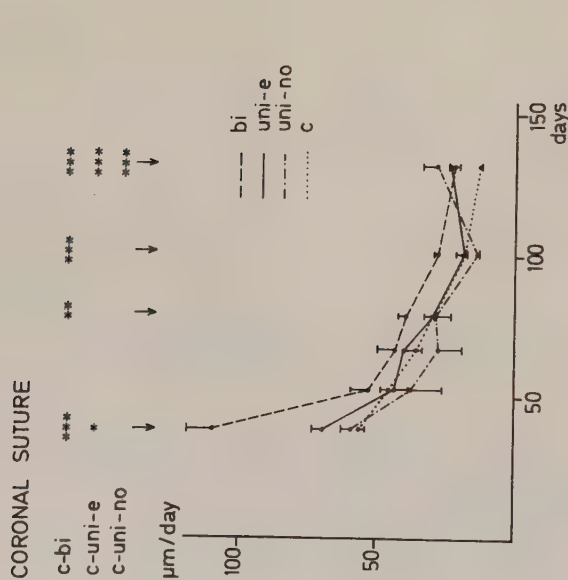


Fig. 6 Growth rates of coronal sutures. Bilateral (bi): n=12 (sides), unilaterally extirpated side (uni-e): n=4, unilaterally non-operated side (uni-no): n=3, controls (c): n=20. For significance levels, see Fig. 5.

INTERFRONTAL SUTURE

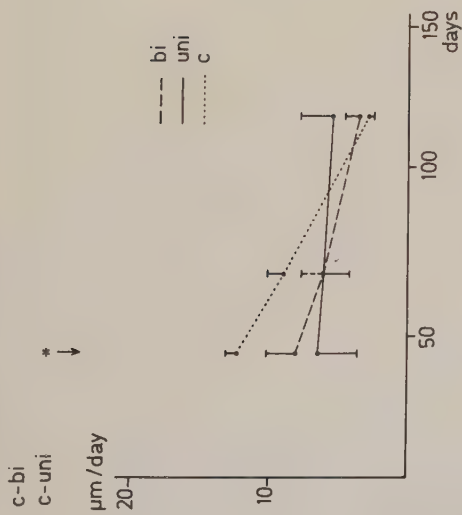


Fig. 7

INTERPARIETAL SUTURE

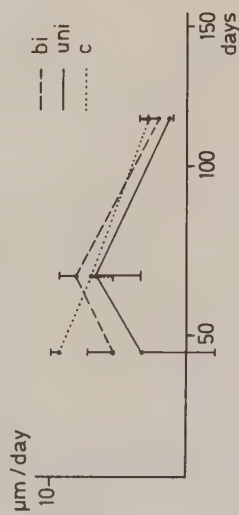


Fig. 7

CALVARIAL VOLUME INCREASE

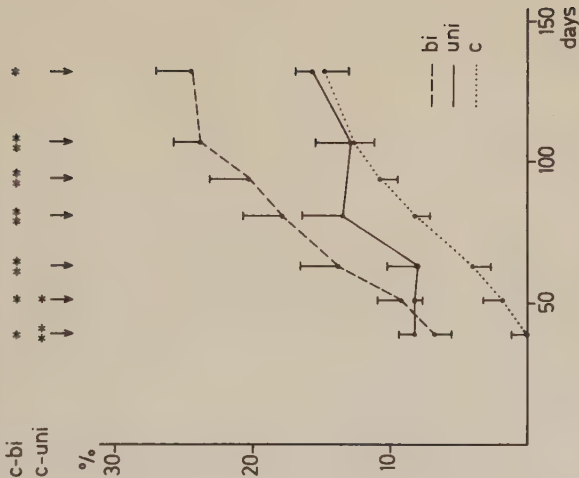


Fig. 8 Volumetric expansion of the polyhedron constructed of bone markers in calvarial bones as related to initial volume. Bilateral: n=4 (rabbits), unilateral: n=4, controls: n=9. For significance levels, see Fig. 5.

ROENTGEN STEREOPHOTOGRAMMETRIC ANALYSIS OF RESTRICTED PERIODS OF NEUROCRANIAL SUTURAL IMMOBILIZATION IN RABBITS

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Running head: Effects of restricted neurocranial sutural immobilization.

Key words: Cranial suture, Craniostenosis, Metallic implant, Roentgen
stereophotogrammetry, Skull growth, Tibial growth.

SUMMARY

The effect of temporary sutural fusion on craniofacial growth was studied biometrically. Four week old male New Zealand white rabbits were used. Tantalum bone markers were implanted in the cranial vault, and the coronal suture immobilized with isobutyl-2-cyanoacrylate adhesive. Linear craniectomy was performed after 2 or 6 weeks of sutural growth restriction (4 animals in each group). The animals were followed for approximately 17 weeks. Early craniectomy resulted in greatly increased coronal suture bone separation (175 % of control growth rates in peers and 125 % of maximal control rates), while growth after late craniectomy showed an age-dependent lower rate, but still the overshoot markedly surpassed controls (210 % of control rates in peers and 65 % of maximal control growth rates). Overall, both exhibited overcompensation in coronal sutural growth. A rapid compensation of adjacent sutures to temporary growth restriction, as well as tendencies towards spontaneous correction following release of growth inhibition was demonstrated. Hereby the total antero-posterior growth of the combined frontonasal and coronal sutures nearly equaled that of control animals. Following linear craniectomy volumetric calvarial expansion increased considerably in both groups, due to compensatory coronal sutural growth, but probably also to spatial bone rearrangements. Based upon the findings, the length of the period of sutural immobilization seems critical both to longitudinal and volumetric growth as well as subsequent compensatory activity after surgical correction.

INTRODUCTION

Craniosynostosis designates premature closure of cranial sutures, inhibiting normal craniofacial growth. Its pathogenesis, and thereby treatment, is still disputed. Restriction of skull growth may, though not necessarily, result in decreased cranial cavity volume, intracranial hypertension and compensatory skeletal deformations, either in the free areas of the vault or in the weak points of the cranial base (58).

In order to experimentally study aspects of premature sutural closure, artificial fusion has been accomplished by several means. Metal clips (35),

bone grafting (18, 23, 30, 56, 57), periosteal application (8, 9, 49), cyanoacrylate adhesive (13, 20, 42, 43) and periosteal stripping (40) have been used. Cyanoacrylate adhesive offers the advantage of an exact immediate immobilization with development of an ectocranial bone bridge (20, 43) and skull base deformations similar to those associated with synostosis in man (42, 43).

Most surgeons empirically recommend early surgical correction of cranosynostosis (e.g. 10, 24, 33, 35, 37, 53, 58) for which experimental support has recently been presented in neonatal rabbits (42). Clinically, though, craniostenosis may not be apparent until the child is more than one or two years old. In these patients, early routine examinations do not reveal any abnormalities associated with the calvarial sutures. Evidently, there are possibilities of "late" premature sutural fusion; that also of obscure etiology and development. Also, the compensatory reactions of non-fused cranial vault regions are unclear, both in relation to duration of sutural growth restriction and the following surgical intervention. Thus, the purpose of this investigation was to elucidate these intricate issues in the rabbit, using highly accurate roentgen stereophotogrammetric analysis with the aid of metallic implants (RSA; Selvik) (52), and thereby assess linear and volumetric growth changes before and after linear craniectomy following coronal suture immobilization. Furthermore, to detect any eventual temporary variations in somatic growth due to surgically induced health disturbances, tibial growth was followed parallel to craniofacial development.

MATERIAL AND METHODS

Experimental animals

Eight 4 week old (30 ± 2 days) male New Zealand white rabbits were used. The animals were divided into two experimental groups of 4, based on the time of surgical release of the immobilized coronal suture. The control group (10 animals) was identical to the one presented in a companion paper (6). The animals were treated and followed as described in the aforementioned paper.

Experimental suture immobilization

The anaesthetic and surgical procedures (apart from sutural immobilization), the roentgen stereometric procedure, analysis of data, and macroscopic investigation have been described previously (4, 6).

After identification of calvarial sutures and subsequent implantation of 0.8 mm tantalum bone markers, small strips of periosteum (3 x 6 mm; Fig. 1) were excised from the parietal and frontal bones parallel to the coronal suture, leaving the periosteum across the suture intact. A dental drill was used to roughen the exposed bone, after which a layer of isobutyl-2-cyanoacrylate adhesive (Bucrylat[®], Ethicon) was bilaterally applied across the coronal suture, to obtain sutural immobilization (20, 43). The sagittal suture was carefully avoided. The adhesive was allowed to polymerize and a topical antibiotic powder (Nebocetin[®]: Neomycin 0.33 g, Bacitracin 25.000 IU) was sprinkled over the open wound, which was subsequently closed (4, 6).

Experimental craniectomy

After 15 (group I) or 42 (group II) days of sutural immobilization, the coronal suture area was re-exposed and linear craniectomy (Fig. 1) (= bilateral suturectomy; 6) performed.

Stereo examinations

Initial stereoroentgenograms were obtained on the day of implantation and adhesive application (age 4 weeks). Examinations occurred at weeks 6, 8, 10, 14, 16, 22. Group I was also examined week 9, and group II week 12.

To check effectiveness of coronal suture growth restriction in group II, the immobilization period (week 4 to 10) was treated as a single time-period.

To reduce the influence of technical errors (4) on the growth rate values and to match the periods of immobilization, the sagittal suture was studied for weeks 4, 6, 10, 14, and 22.

Tibial growth

To estimate the effect of craniectomy on somatic growth, and thereby

obtain a measure of general health (3, 11), tantalum balls (0.5 mm in diameter) were bilaterally implanted in the proximal and distal tibial epiphyses in 13 animals (8 experimental and 5 control animals). This was done under fluoroscopic control, by means of an angiographic needle with an outer diameter of 0.97 mm at a somewhat later date than cranial implantations (36 ± 3 days), due to equipment problems. The same analgesic was employed as described above.

RESULTS

Macroscopic findings (Fig. 2 a and b)

In both groups, the overall calvarial bone and suture morphology was comparable to controls, excepting the craniectomy sites. All defects remained open down to the temporal bones, some even increasing somewhat in size. Animals subjected to prolonged immobilization attained an upward displacement of the parietal bones, most pronounced rostrally.

Lateral deviation of the snouts (group I: range 0° to 3.5° , mean 1.1° ; group II: range 0° to 2.0° , mean 1.1°) were comparable to controls (range 0° to 2.0° , mean 1.1°).

The frontonasal suture (Fig. 3 a)

Rabbits subjected to antero-posterior coronal suture growth restriction for 15 or 42 days generally exhibited a pattern of irregular rapid growth rate deceleration similar to controls. The growth curves intermittently paralleled each other, with minor rate variations relative to one another. Release of coronal suture growth restriction resulted in periods of reduced growth rates for about 2 (group I) to 3 (group II) weeks, thereafter returning to control rates.

The coronal suture (Fig. 3 b)

Coronal sutures immobilized by cyanoacrylate adhesive exhibited rapid cessation of normal calvarial growth. Surgical release of the immobilized sutures at age 6 or 10 weeks resulted in an immediate significantly accelerated separation of the frontal and parietal bone margins. Thus, immediately after early craniectomy, the growth rate increased 175 % over controls, and 125 % over the controls maximal rate during the investigation

period. Following late craniectomy a sudden initial sharp increase in growth rate was also observed, exceeding control equivalent values by 210 % and the maximal rate of controls by 65 %.

Generally, the separation rate declined successively, experimental groups exceeding controls considerably.

The sagittal suture (the internasal, interfrontal and interparietal sutures) (Fig. 3 c, d and e)

Neither antero-posterior growth restriction nor its surgical release affected growth at the internasal or interparietal sutures. On the other hand, the initial interfrontal sutural growth rate in both groups almost reached twice the control rate. Thereafter, growth values approached controls, only to finally rebound to double that of controls.

Prolonged antero-posterior restriction caused a reduced bone separation in the interparietal suture following craniectomy. Considering the small group size ($n = 3$), it can be speculated that a larger sample might have more clearly demonstrated this reduction.

Volume (Fig. 4)

During the short and prolonged growth restriction periods, calvarial volume remained similar to controls. Only the short immobilization resulted in a dramatic reaction to craniectomy. This group ultimately obtained a final volume exceeding controls by 190 %. Prolonged immobilization finally exceeded controls by approximately 70 %. No levelling off was observed during the final intervals.

Mean total growth (Table I)

The mean total growth at the frontonasal suture decreased following short neurocranial antero-posterior growth restriction. As for the coronal suture, both groups remarkably increased their mean growth rates. Nevertheless, total antero-posterior growth, comprising the frontonasal and coronal sutures, amounted to nearly identical growth rates in both experimental groups. Total sagittal sutural growth was similar to controls with the exception of increased bone separation at the interfrontal suture following prolonged growth restriction.

General health control

The tantalum bone markers implanted bilaterally in the tibial epiphyses demonstrated a continually diminishing rate of separation. Surgical procedures affected tibial growth differently (Fig. 5). Generally tibial tantalum ball implantation initially seemed to reduce growth slightly.

Early linear craniectomy caused a pronounced brief fall in growth rate, but although the same tendency occurred following late craniectomy, the latter depression was not significant. Nevertheless, a subsequent period of compensatorily increased growth was registered.

DISCUSSION

Experimental model

The present study confirmed the effectiveness of isobutyl-2-cyanoacrylate adhesive to mechanically immobilize sutures. Thus, experimental investigation on localized growth retardations and the subsequent effects of surgical correction are facilitated. Of interest is the fact that cranial vault linear growth inhibition perpendicular to a suture is combined with adjacent compensatory growth in a direction parallel to the immobilized suture (Virchow's law).

Recently, several reports on the importance of mechanical constraint of the fetal head in the development of premature sutural fusion have been presented (24-27, 31). Correspondingly, ectocranial bony fusion has been shown to occur by the constraint of cyanoacrylate adhesive (20, 43). Also, subsequent changes in angulation between bones similar to those noted in human craniosynostosis have been observed (42, 43). This model would, accordingly, be relevant to experimental studies in this field.

Careful control of marker stability is of major importance when using metallic markers for growth registrations. If instability is registered and technical errors can be excluded, the unstable marker is omitted. This is a prerequisite for the methods high accuracy (4, 52). RSA permits the presentation of growth rate diagrams instead of linear changes. Thus, by a single diagram, information on total growth (area below the curve), the rate itself, and the change of rate (the slope of the curve) is obtained

in contrast to only the first two parameters on conventional diagrams.

Calvarial growth following sutural immobilization

In the present study no tendency towards interruption of the cyanoacrylic bridge was observed, even in the 42 day long restricted growth interval. Foley and Kokich (20) found tissue disruption with subsequent encapsulation of the foreign material within 30 days, suggesting a temporary effectiveness of the growth restriction. Evidently, this is not supported by our results as neither coronal sutural growth nor a neurocranial volumetric increase comparable to controls were registered.

Premature closure of the coronal suture is a major trauma to normal skull expansion. Sutural fusion involving only a small segment may spread to involve other sutures as well as follow the entire length of the coronal suture due to its continuity in the skull base (7, 10, 16, 21, 51, 53, 54). Often neurocranial and facial skeletal aberrations are coupled (19, 38, 42, 43, 58).

Nevertheless, after immobilizing neurocranial antero-posterior growth, the frontonasal suture was found to retain its growth pattern despite contact with the frontal cerebral lobes. This is contrary to Babler et al. (13), who observed frontonasal sutural growth to decrease during this period.

On the other hand, we observed the neurocranial interfrontal suture to substantially increase its growth rate compensatorily in both experimental groups. This contradicts a radionuclide investigation by Gates and Dore (21), who found the expansile growth rates of non-fused sutures to be normal or only slightly accelerated. However, this strongly suggests that one area of disharmonic growth causes time-dependent disturbances in regions and structures adjacent to it (12, 13, 34, 41).

Calvarial growth following linear craniectomy

The aim of surgical intervention is optimal correction of cranial deformity and the disproportion between the volume of the normally expanding brain and capacity of the cranial cavity. Although our animals have passed the neonatal period, which entails a successive reduction in neural tissue

growth (29), the release of immobilization caused a dramatic bone separation at the coronal suture. Even late craniectomy resulted in postoperative growth rates markedly surpassing the maximal observed control rate. Bone separation even reached a total level almost identical to that following removal of the normal coronal suture at age 4 weeks (6). This consequently comprises an overcompensation.

By studying unmanipulated sutures, insight into the sutural compensatory ability was again attained. Following craniectomy neurocranial antero-posterior growth resulted in normalized bone separation along the interfrontal suture. Also, nearly discontinued interparietal sutural growth was registered after late craniectomy. Here the converse of Virchows law was observed, probably a buffering effect to reduce intracranial volumetric fluctuations.

Interestingly, a significant immediate post-craniectomy growth reduction at the frontonasal suture was noted in both groups. Animals subjected to early craniectomy did not compensate for this growth reduction during the remaining period (13). As the frontonasal sutural growth had already diminished considerably at the time of late craniectomy, surgical intervention did not markedly affect total longitudinal growth. Sutures which normally exhibit considerable bone formation thus seem to show greater postoperative responses (12, 13, 34).

When comparing the total longitudinal antero-posterior growth rates (comprising the frontonasal and coronal sutures), these amounted to rates almost identical to controls. Thus, increased bone separation at one suture is compensated by reduced separation at another, supporting the passive nature of growth at these sutures.

However, another explanation can be offered. In a previous paper (3) tibial and craniofacial growth were registered simultaneously. During a brief period of nutritional deficiency it was found that frontonasal sutural growth paralleled a pronounced decline in tibial growth. On the other hand, coronal sutural growth was mainly unaffected. Thus, presently observed reductions in frontonasal sutural growth may be explained by a temporary health decline due to surgical trauma, as demonstrated by the tibial growth diagram.

Following craniectomy, both experimental groups showed an increased calvarial polyhedral volume (for further comments on methodological aspects, see the companion paper) (6). Bertelsen (15) argued that volumetric growth in coronal synostosis is the result of shortening of the anterior section of the cranial base, internal bone resorption, external deposition and deformities in the skull due to increased intracranial pressure, as judged by the digital markings, the bulging of the bregma and the temporal regions, and the downward displacement of the floor of the anterior and middle cranial fossae. This does not fully explain our results. These are partly explained by the macroscopically verified upward rotation of the parietal bones following prolonged immobilization. However, the cranial vault width remained identical to controls.

Considering that the intracranial volume should only increase slightly (29), it may be speculated that a spatial readjustment of cranial bones might have taken place (one rotational axis lying parallel to the measured cranial vault width). Also, continual rotational activity of calvarial bones normally takes place during the cranial vault growth period (5).

Interestingly, craniectomized animals displayed a calvarial volumetric increase substantially greater than controls. This overcompensation thus hardly implies a major real increase in intracranial volume, but rather reflects the high potential for spatial bone readjustment even during abnormal cranial growth (13, 39).

This study demonstrated the emergence of skeletal aberrations despite the animals being older than neonatal. Speculatively, this stresses the early detection of as well as extended surgical release of sutural growth restrictions in order to enable a rapid return to normal volumetric relations and thereby avoid unfavorable vectorial influences on calvarial growth.

Determination of general health

General health, as evaluated by tibial epiphyseal marker separation, was definitely affected by craniectomy and to a lesser degree by bone marker implantation.

Many studies have shown that externally imposed non-health, such as

nutritional deficiencies, delay long bone growth (review: 32). Riesenfeld (47) observed long limb bone growth to be significantly reduced relative to the length of the neurocranium after severe reduction of dietary proteins and calories. Thus, postoperatively reduced food intake might be expected to affect at least long bone growth. Other attempts to explain postoperative growth reduction includes acute infection (1, 36), posttraumatic growth inhibition (28, 61), postoperative general impairment of blood flow (14, 22, 28) or endocrine influences (48, 59, 61).

Neurocranial growth is considered to closely reflect that of the brain. In the rat, nutritional deficit is known to slightly affect brain growth (2, 17, 44, 46, 50, 55, 62), as well as stunt craniofacial growth; the neurocranium less than the splanchnocranium (44-47, 60), which results also seem applicable to rabbits (3).

By using tibial markers, peroperative changes in long bone growth were detected and general growth thereby evaluated. Evidently, any brief post-craniectomy stunting of neural tissue expansion did either not markedly influence growth at the sutures studied, or such was concealed by the dramatic release of sutural growth restriction. Nevertheless, tibial markers proved a sensitive indicator of general health, enabling detection of surgical and thus external effects on experimental results.

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TABLE I. Mean longitudinal growth rates per day. Significance levels (Student t-test) indicated:

* $p \leq 0.05$;
 ** $p \leq 0.01$;
 *** $p \leq 0.001$

	<u>G r o w t h - r e s t r i c t i o n :</u>				<u>C o n t r o l s :</u>			
	<u>S h o r t</u>		<u>L o n g</u>		<u>μm/day</u>		<u>SEM</u>	
	μ m/day	SEM	n	μ m/day	SEM	n	μ m/day	SEM
Internasal suture	16.4	1.5	3	16.3	1.0	2	20.6	3.3
Frontonasal suture	136.7**	5.6	7	145.5	4.7	6	142.3	11.1
Interfrontal suture	8.2	0.9	4	9.4*	1.4	4	6.5	0.6
Coronal suture	46.0***	1.5	8	38.8***	2.7	8	28.4	0.8
Interparietal suture	5.2	1.0	4	3.8	0.8	4	5.3	0.7
Cranial vault width	19.8	2.9	4	19.3	3.1	4	19.3	2.2

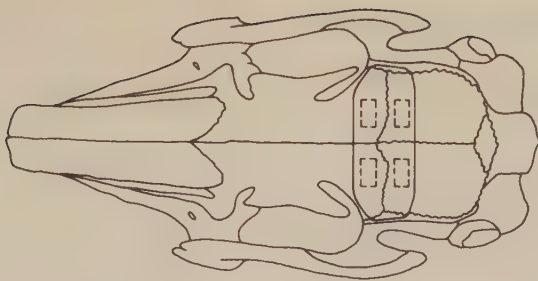
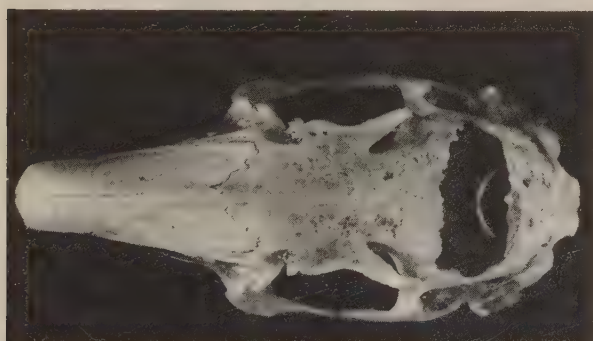
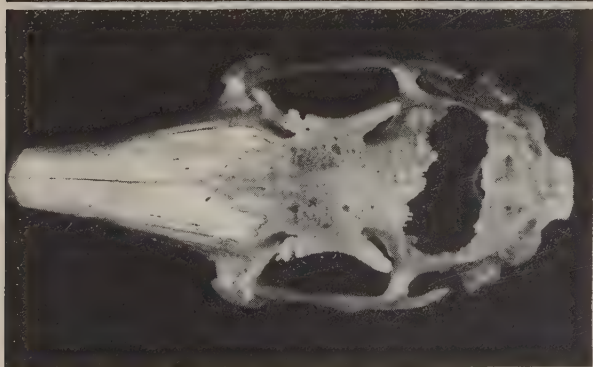


Fig. 1 Dorsal view of the experimental sites. Areas of isobutyl-2-cyanoacrylate adhesive (---) and of craniectomy (-----) indicated. The adhesive bridges the coronal suture.



a



b

Fig. 2 Photographs showing degree of bone regeneration after craniectomy. a/ early craniectomy, b/ late craniectomy.

FRONTONASAL SUTURE

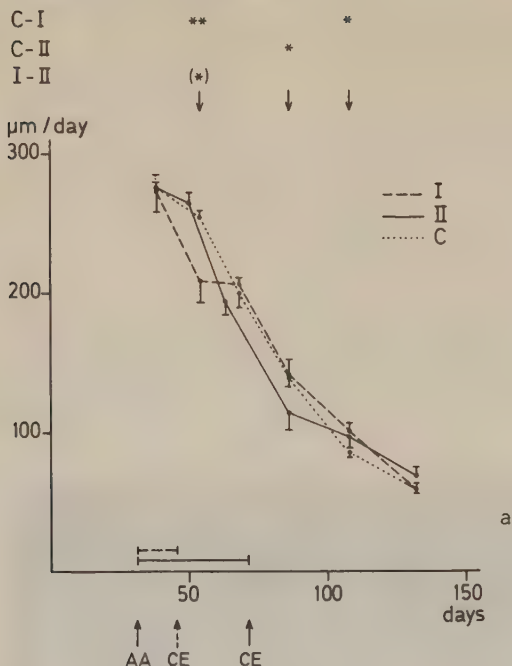


Fig. 3 Growth rates of investigated sutures. Generally short and prolonged immobilizations are represented by equal numbers of sides. a/ frontonasal suture: $n=7$ (sides), controls: $n=17$. b/ coronal suture: $n=8$, controls: $n=20$. c/ internasal suture: $n=3$ (rabbits), controls: $n=8$. d/ interfrontal suture: $n=4$, controls: $n=10$. e/ interparietal suture: $n=4$, controls: $n=10$. AA = adhesive application. CE = craniectomy. The standard error of the mean (SEM) is indicated by brackets. Significance levels (Student t-test) indicated: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$. Significant changes were not registered in figs. c and e.

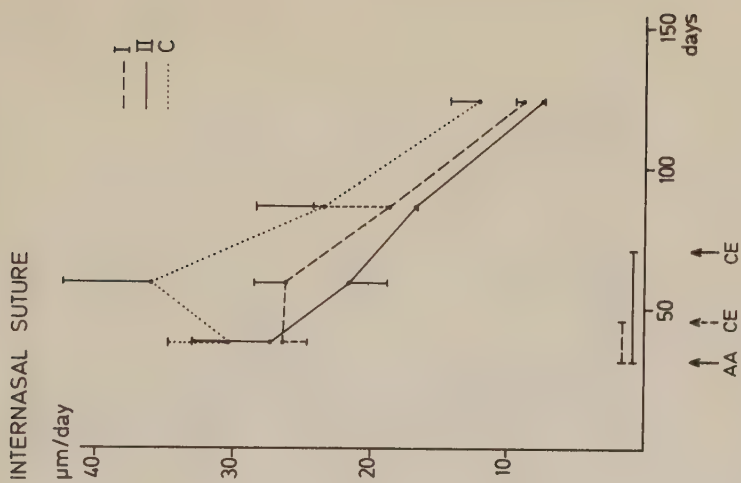


Fig. 3 c

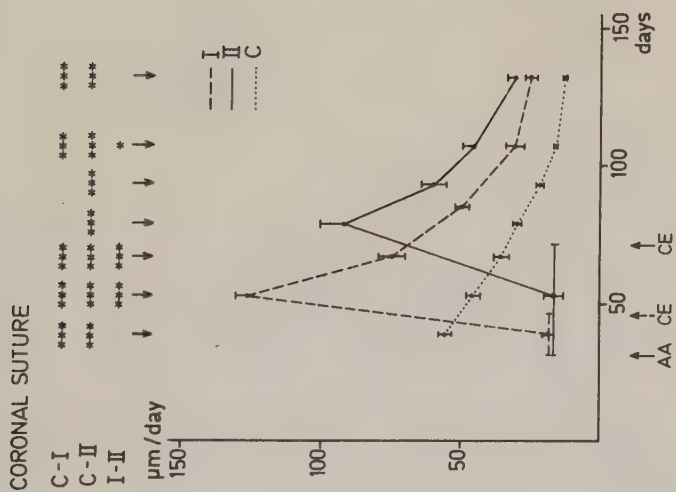
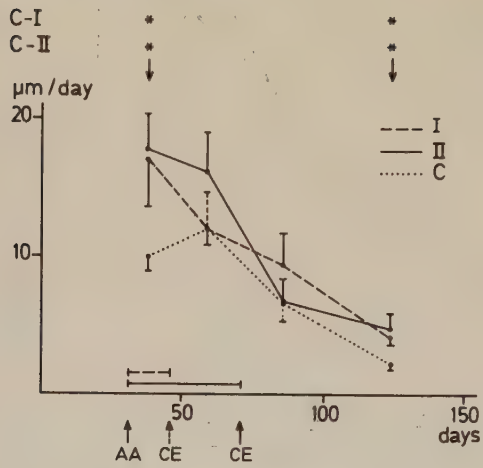
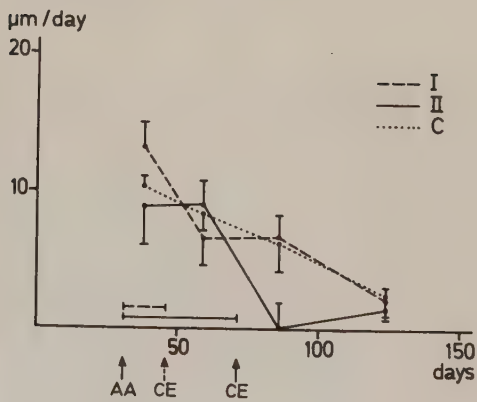


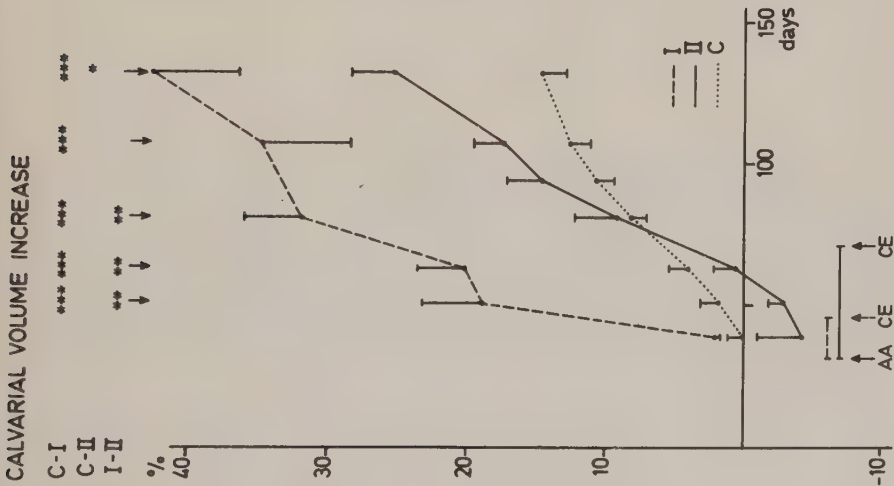
Fig. 3 b

INTERFRONTAL SUTURE



INTERPARIETAL SUTURE





Volumetric increase of polyhedron constructed of bone markers in calvarial bones. Group I and II: n=4 (rabbits), controls: n=9. For significance levels, see Fig. 3.

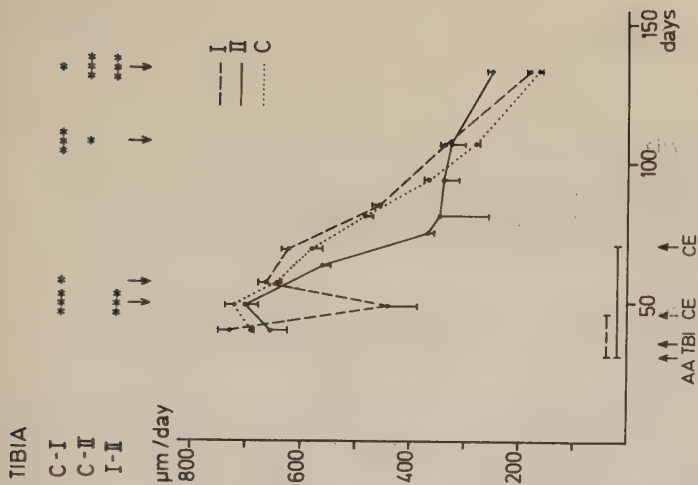


Fig. 5 Combined growth rates of proximal and distal tibial epiphyses. TBI = tibial bone marker implantation. Group I and II: n=8 (sides), controls: n=10. For significance levels, see Fig. 3.

Pattern of Membranous and Chondral Bone Growth. A Roentgen Stereophotogrammetric Analysis in the Rabbit

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Key Words. Growth · Bone · Craniofacial development · Tibia · Rabbit · Metallic implant · Roentgen stereophotogrammetry

Abstract. To elucidate longitudinal tubular bone growth patterns in relation to craniofacial development, nine 4-week-old male New Zealand white rabbits received tantalum implants in the calvarial bones and in the proximal and distal tibial epiphyses. The animals were examined by roentgen stereophotogrammetry. The rapid growth rate deceleration pertained to all growth regions studied, except the sagittal suture. A brief period of under-nutrition highlighted the parallel in growth rates between the frontonasal suture (associated to the facial skeleton) and the tibia. The neurocranial coronal suture exhibited a different growth pattern, presumably related to neural tissue expansion, which was also observed to rapidly react to nutritional deficiency. Tibial growth was found to sensitively reveal influences of somatic development. Hence, it might be used to optimize growth evaluations.

Introduction

The calvarial and tubular bones represent two different modes of bone formation, called intramembranous and endochondral ossification, respectively. Intramembranous bone growth proceeds by sutural and periosteal (pericranium and dura mater) bone deposition. Long bone growth, on the other hand, is due to cartilaginous tissue growth at their extremities and to the

periosteal/perichondrial modelling of their surfaces.

Longitudinal bone growth has been studied by a variety of methods, one being implant markers [review *Sarnat and Selman*, 1978]. *Dubreuil* [1913] is credited for combining metallic implants and radiographic measurements, which were used to study rabbit tibial growth. This technique was later applied to the rabbit [*Levine*, 1948] and human [*Björk*, 1955] craniofacial skele-

ton. Furthermore, long bone and craniofacial growth measurements have been combined due to the close association in timing of the pubertal somatic growth spurt and of facial dimensions. Björk [1972] introduced the use of endochondral bone growth, as estimated by hand radiographs, as an indicator of pubertal growth spurt onset in orthodontic treatment.

The general condition, or health, is known to influence bone growth. Thus, to make growth recordings representative, minimization of external influences, such as illness, are crucial. Weight, though, often used as an indicator of health, has been found to inadequately reflect individual animal health in studies of craniofacial growth [Alberius and Selvik, 1982]. Hence, normal growth fluctuations cannot be distinguished from occasional sickness. Also, Hansson [1967], using the tetracycline method, observed no correlation between rabbit long bone growth and weight.

Another perspective was added by Aronson et al. [1978]. When studying daily rabbit tibial growth by roentgen stereophotogrammetric analysis (RSA) [Selvik, 1974], they even observed decreased growth rates following temporary diarrhoea. Evidently, the high accuracy of RSA enabled detection of slight growth variations. Therefore, it was decided to elucidate whether tibial growth could be used as a control of animal health in craniofacial studies.

Material and Methods

Animals

Nine 4-week-old (30.0 ± 0.9 days; mean weight 0.665 ± 0.132 kg) male New Zealand white rabbits were used. The animals were kept at $20\text{--}21^\circ\text{C}$ (40%

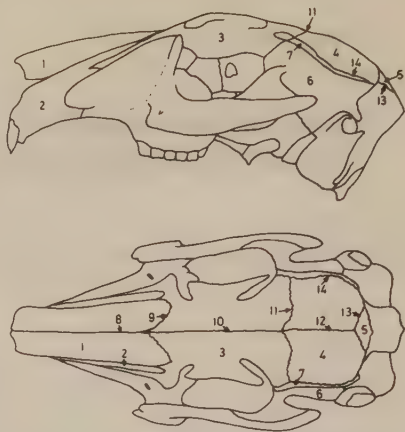


Fig. 1. Relevant anatomy. Bones: (1) nasal, (2) premaxilla, (3) frontal, (4) parietal, (5) interparietal, and (6) temporal. (7) Temporal line. Sutures: (8) internasal, (9) frontonasal, (10) interfrontal, (11) coronal, (12) interparietal, (13) lambdoidal, and (14) temporal.

relative humidity), with light from 6 a.m. to 6 p.m., and with standard pellets and water provided ad libitum.

Following examination at age 7 weeks (see below), a new water-distributing system was installed, leading to a 3- to 4-day refusal to drink and thereby a decrease in food intake (about $1/3$ of each when compared to successive normal amounts). Thereafter no complications were observed. The animals were followed until age 21 weeks (145.9 ± 5.7 days), since, according to Engdahl [1972], their growth curve is shown to level off at an age of 5 months.

Operative Technique

The animals were anaesthetized by intramuscular neuroleptanalgesia (Hypnorm® vet.: Fluanizon and Phentanyl, 10 and 0.2 mg/ml, respectively; 0.6 ml/kg body weight). The head was shaved and the surgical area cleaned with alcoholic Chlorhexidin (5 mg/ml). A para-midline skin incision from the parietal to the nasal region was made. The skin and underlying tissues were reflected laterally and calvarial sutures identified (fig. 1). Tantalum bone markers (0.8 mm in diameter) were implanted in the nasal, frontal and parietal bones,

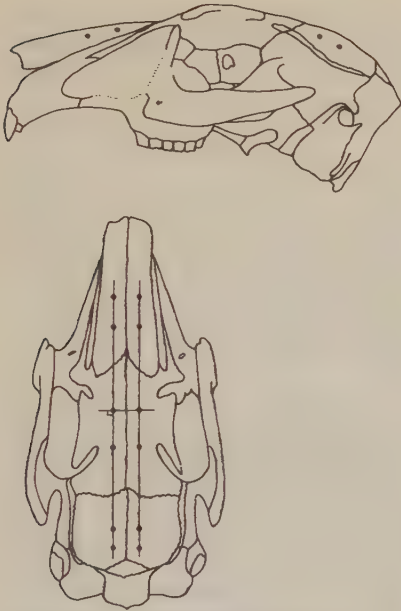


Fig. 2. Tantalum bone marker positioning.

using a specially constructed instrument [Aronson et al., 1974]. The markers were implanted along bilateral rostral-caudally oriented lines parallel to the median plane; the connecting line between each bilateral pair at a right angle to the midline (fig. 2). Care was taken to minimize periosteal manipulation so as not to influence bone production. After the implantation of bone markers the incision was closed with interrupted 4/0 Ethicon sutures and covered with Nobecutan® spray. Thereafter, tantalum balls (0.5 mm in diameter) were percutaneously, under fluoroscopic control, bilaterally implanted in the proximal and distal tibial epiphyses by means of an angiographic needle with an outer diameter of 0.97 mm. The animals were left to recover on a warm electric blanket.

Stereometric Examinations

Initial stereoroentgenograms were obtained on the day of implantation (age 4 weeks) and at ages 6, 7, 8, 9, 10, 12, 14, 16, and 21 weeks.

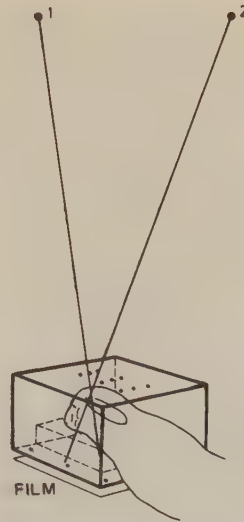


Fig. 3. Roentgen stereoexamination. The rabbit head is placed on a support in the calibration cage and is simultaneously radiographed by two roentgen tubes (focus 1 and 2). Cage reference markers in the upper and the lower plane indicated (•).

Roentgen Stereophotogrammetry Apparatus

The animals were examined in a glass calibration cage supplied with tantalum reference markers (fig. 3). Two simultaneously exposing roentgen tubes were positioned with an approximate focus-to-focus distance of 0.4 m and a focus-to-film distance of 1.0 m. The maximal radiation dose used for one examination was 0.6 mGy [Alberius and Selvik, 1982].

Analysis of Data

The films were digitized in a precision instrument for aerial photogrammetry (Wild Autograph A8). The 3-D coordinates of the bone markers were obtained by computer processing. The distance between the bone markers and distance changes with time were calculated, the latter were taken as the longitudinal growth. The rate of distance change was related to the mean day in the investigation interval and expressed in $\mu\text{m/day}$. Statistical evaluations were conducted, the mean rates

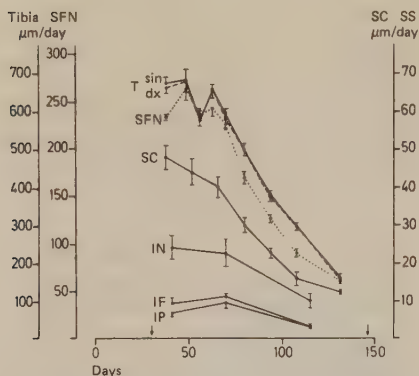


Fig. 4. Craniofacial and tibial growth rates. T = tibia, SFN = frontonasal suture, SC = coronal suture, SS = sagittal suture: internasal (IN), interfrontal (IF), and interparietal (IP) suture. Arrows represent start and cessation of investigation period. Brackets indicate standard error of the mean (SEM).

with related standard error of the mean (SEM) are presented. Methodological errors were 21.0 μm for each growth registration [Alberius and Selvik, 1982].

To optimize the evaluation of coronal suture growth, analyses were conducted for minimally 2-week intervals, thus omitting weeks 7 and 9. The sagittal suture, whose growth between succeeding measurements approached the methodological error, was analyzed for weeks 4, 7, 12 and 21.

Results

Craniofacial Growth (fig. 4)

Frontonasal Suture. Following the initial value (232.5 $\mu\text{m}/\text{day}$) a rapid almost linear growth rate decrease was observed during the observation period. Exceptions were the second and the fourth interval, where a 12.7 and a 2.5% increase relative to the preced-

ing rate values were registered. The final value measured implied a growth decrease to one-fourth (26.3%) to have occurred during the interval studied.

Coronal Suture. The neurocranial coronal suture exhibited a slightly less pronounced deceleration in bone separation compared to the frontonasal suture, which is closely related to the facial skeleton. Neither marker implantation nor decreased nutritional intake seemed to markedly affect the sutural expansion. The finally registered rate value amounted to 25.5% of the initial value.

Sagittal Suture. The nasal bones, part of the facial skeleton, separated with a successively reduced rate during the investigation, yet constantly greatly exceeding the rate of the caudal sagittal suture. On the contrary, the neurocranial part of the sagittal suture (the interfrontal and the interparietal suture) showed a different growth pattern. Here, a slight increase in rate values was observed following the initial interval. Overall, the sagittal sutures growth rate diminished less rapidly (42.5, 33.7, and 46.4% for the internasal, interfrontal and interparietal sutures, respectively) than those of the antero-posteriorly growing sutures.

Tibial Growth (fig. 4)

A very rapid marked fall in growth rate (initial growth rates: left side 673.5; right side 660.6 $\mu\text{m}/\text{day}$) was registered, the right and left sides constantly closely following each other. Exceptions were the same as for the frontonasal suture; the first interval was followed by an increase of 2.0%, while the third by 13.1% (mean values of left and right sides). The last interval studied exhibited a growth of 23.9% compared to the first one.

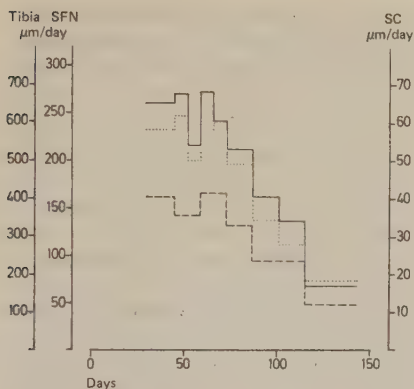


Fig. 5. Separate illustration of tibial and antero-posteriorly growing craniofacial sutures' growth rates for one rabbit. T = tibia (—), SFN = frontonasal suture (.....), SC = coronal suture (---).

Individual Comparison (fig. 5)

Tibial and antero-posteriorly growing sutures for 1 rabbit are shown to illustrate relations and interactions of individual growth regions. Tibial and frontonasal suture growth tended to follow each other throughout the investigation. Marker implantation only slightly affected growth, while nutritional deficiency greatly reduced growth rates. Also, the coronal suture markedly reacted on reduced and restituted food and water intake in this animal.

Discussion

Bone Marker Implantation

To optimize the sensitivity of tibial growth recordings, tantalum balls were

implanted in both epiphyses. Thereby influences from changing ratios of proximal and distal growth plates [review *Hansson*, 1967] were avoided. Also, the metaphyseal bone has been reported to not completely stabilize bone markers [*Aronson*, 1976].

At implantation, the tibial periosteum was penetrated by an angiographic needle containing the metallic marker. Periosteal manipulation has earlier been reported to slightly affect growth. *Hansson* [1967], making a V-shaped incision of metaphyseal periosteum, observed a postoperative increase after the first day. *Crilly* [1972] found transverse sectioning of diaphyseal periosteum to stimulate longitudinal growth, while the effect of longitudinal incision was negligible. Thus, the direction and extent of periosteal manipulation seems to be a noteworthy factor.

In this study, the needle, having an outer diameter of 0.97 mm, caused a minor circular periosteal resection. A few of the implantations, though, were not immediately successful, resulting in greater periosteal trauma. *Aronson and Hansson* [1976] investigated, by the tetracycline method, the same implantation technique as used for craniofacial implantations in this study [*Aronson et al.*, 1974], using tantalum pins. An initial minute local growth stimulation was only observed following larger marker (0.5×1.5 mm) implantations day 1 and 2 after insertion. Nevertheless, an initial instability before bone adhesion to the marker surface [*Alberius*, 1982] is to be expected [*Rune et al.*, 1979, 1981; *Bylander et al.*, 1981]. Thus, it may be concluded that the initially reduced growth probably does not result from the marker implantation per se, but rather reflects peroperative occurrences, e.g. variations in water and food

intake, postoperative infections [Acheson and Macintyre, 1958], general growth stunting [Wray and Goodman, 1961; Hansson, 1967; Hedström, 1969], or reduced blood flow [Gelin, 1956; Wray and Spencer, 1960; Bergentz, 1961; Wray and Goodman, 1961; Sundén, 1967; Persson, 1968].

As for craniofacial implantations these have been discussed elsewhere [Alberius and Selvik, 1982].

Longitudinal Bone Growth

During the period studied, age 4–21 weeks, tibial growth rates were observed to fall dramatically with age. An almost linear decrease followed the period of undernutrition. Heikel [1959/60] on the other hand, found a successive tapering off primarily after 100 days of age.

Investigations on major long bone growth, performed on different species, have been mostly restricted to one bone end. Furthermore, differences in the duration of growth periods and skeletal development between species [Heikel, 1959/60] make comparisons difficult. Rabbits, though, generally show high growth rates, making them suitable for studies on long bone growth [review Hansson, 1967]. Hansson [1967], using the tetracycline method in studies of rabbit longitudinal long bone growth between day 20 and 70, observed highest growth rates for both the proximal and distal tibia at day 30. These combined values amounted to 1,050 $\mu\text{m}/\text{day}$, markedly exceeding our rates, after which they successively approached growth rates registered in this study. Yet, Hansson et al. [1974], investigating diurnal growth rates in 5-week-old rabbits using the same technique, obtained a mean daily growth of 726 μm , which is similar to our values for that

age. Also, Brodin [1955], Trueta and Amato [1960], Heikel [1959/60] and Aronson et al. [1978] reported lower tibial growth values than Hansson [1967]. Furthermore, it must be realized that growth values are related to a multitude of factors: species, age, growth region, different parts of the growth plate and also influences by hereditary factors and nutrition [review Hansson, 1967].

As for cranial growth [discussed elsewhere by Alberius and Selvik, 1982], a domination of splanchnocranial growth was observed for the investigated period. This predominance of facial skeletal growth implies an alteration of skull form from klinorhynchal (facial skeleton lies subcerebrally) to orthocranial (facial skeleton pre-cerebrally) as suggested by several authors [e.g. Baer, 1954; Moss, 1958; Pucciarelli, 1981; Engström et al., 1982]. The decreasing antero-posterior growth, comprising the frontonasal and coronal sutures, finally amounted to one-fourth of the initial values. Interestingly, this implies neurocranial and facial skeletal growth decreases to parallel each other, despite different functional matrices [Moss and Salentijn, 1969]. Additionally, the tibial growth fall, also to one-fourth of initial values, demonstrates a parallel deceleration for the studied parameters.

The increased sagittal suture expansion at the midpoint of this investigation indicates that the rapid coronal suture growth fall [Alberius and Selvik, 1982] is compensated somewhat by increased separation at the caudal part of the sagittal suture. This agrees with the still continuing neural tissue expansion [Harel et al., 1972].

The earlier demonstrated differences between left and right sides of cranial vault growth (especially pertaining to frontonasal sutural growth; Alberius and Selvik [1982])

did not seem to be of comparable magnitude and importance in long bone endochondral growth. Here, the left and right tibiae closely paralleled each other, with only minor variations (within the methodological error) relative to each other. This has been previously established [Hansson, 1967].

The separately described rabbit points to the very intimate relation between the tibia and the frontonasal suture during this interval. Growth rate rebounds and great variabilities were observed. The coronal suture, though, governed by the cerebral expansion, exhibited growth less affected by external influences.

Nutrition

Following the short period of nutritional deficiency a pronounced decrease in growth rate was observed, particularly in the tibia, while the coronal suture showed minimal change of rate values compared to normal [Alberius and Selvik, 1982]. As the standard pellets used in the present study contain all necessary ingredients for a complete diet, the reduced food intake included all needed nutritional constituents, hence conclusions as to any specific influence of a food substance are impossible. A subsequent rate rebound, particularly in tibial growth, showed a tendency toward compensatorily increased growth, whose extent was impossible to evaluate due to lack of controls. This agrees with previous studies which have shown nutritional deficiencies to delay bone growth. When malnourished animals are rehabilitated dietarily, bones respond with a catch-up period of compensatory growth [review Himes, 1978]. The degree of catch-up is dependent on bone maturity (age), duration and severity of malnutrition.

As confirmed in this study the skull is

much less affected by food deficit than the rest of the body [Riesenfeld, 1967]. All nutritional stresses, though, are considered to delay craniofacial development [Pucciarelli, 1981], the neurocranium less than the splanchnocranium [Riesenfeld, 1967, 1973; Williams and Hughes, 1978; Pucciarelli, 1980, 1981] which was corroborated in this investigation. Neurocranial length [Riesenfeld, 1973] or breadth [Himes, 1978] are probably the most stable dimensions under conditions producing lowering of body weight.

The brain is known to have a high priority for growth and a relative resistance to undernutrition. Nutritional deficit is known to slightly affect brain growth [Adlard and Dobbing, 1972; McAnulty and Dickerson, 1974; Zamenhof et al., 1974; Dickerson and Pao, 1975; Sara et al., 1976; Pucciarelli, 1980] less than other organs [Riesenfeld, 1967; Srivastava et al., 1972]. Since neural tissue expansion is believed to govern cranial vault growth, it is reasonable to suppose that changes seen in the brain case may reflect changes in brain size [Williams and Hughes, 1978].

In this study, the separately described rabbit showed a definite increase in coronal sutural growth after resumption of food and water intake, indicating a rapid influence on cerebral volume. This may be explained by a prior temporary weight fall of at least the forebrain [Adlard and Dobbing, 1972; McAnulty and Dickerson, 1974; Dickerson and Pao, 1975].

In conclusion, a parameter of health, independent of cranial growth, might be valuable in craniofacial research, weight being too inexact. Tibial growth was found to sensitively reflect influences on somatic development, hence it may be used to optimize the growth evaluations.

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